Ecological factors influencing the distribution and abundance of the saltmarsh mosquito vector (*Aedes camptorhynchus*).

Raylea Maree Rowbottom

Submitted in fulfilment of the requirements for the

Degree of Doctor of Philosophy

Tasmanian Institute of Agriculture

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Declarations by the author

Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, and to the best of my knowledge and belief, no material previously published or written by another person except where due acknowledgement is made in the text of the thesis.

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Statement of co-authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Candidate - Raylea Rowbottom, Tasmanian Institute of Agriculture, University of Tasmania

Author 1 - Geoff Allen, University of Tasmania

Author 2 - Scott Carver, University of Tasmania

Author 3 - Leon Barmuta, University of Tasmania

Author 4 - Phil Weinstein, University of Adelaide

Author 5 – Dhalia Foo, University of Tasmania

* * * * *

Sebastien Comte - University of Tasmania

Publications from the thesis

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Author contributions: RR, SC, and GA conceived the design of the project. RR, SC, GA and PW conducted the field work. RR processed all the laboratory work. LB and RR conducted the statistical analysis. GA oversaw the project and provided conceptual guidance. RR wrote the manuscript with critical feedback from all authors.

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We, the undersigned, agree with the above stated proportion of work undertaken for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Raylea Rowbottom	**Assoc. Prof. Geoff Allen**
Candidate	Primary Supervisor
Tasmanian Institute of Agriculture	Tasmanian Institute of Agriculture
University of Tasmania	University of Tasmania
Date: 30 April 2020	Date: 29 April 2020
Dr. Scott Carver	**Prof. Mike Rose**
Co-supervisor	Interim Director
School of Biological Science	Tasmanian Institute of Agriculture
University of Tasmania	University of Tasmania
Date: 28 April 2020	Date: 29 April 2020

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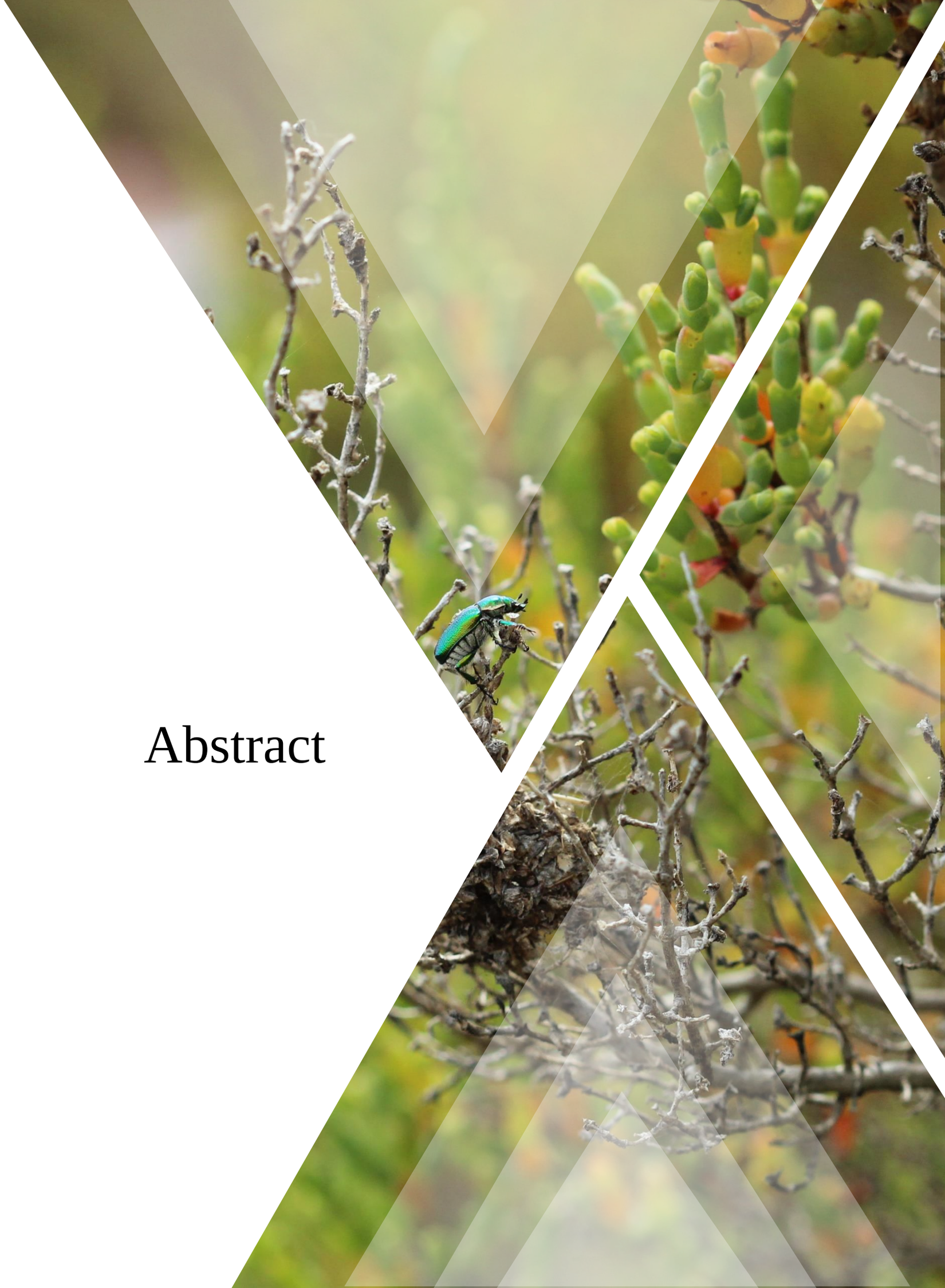


Abstract

Thesis abstract

A major contributor to the transmission of vector-borne disease in many countries are mosquitoes that emerge from saltmarshes. This is particularly the case for Australia, where saltmarsh mosquitoes are arguably the most important for vector-borne infections. The saltmarsh habitat influences the distribution, abundance and success of adult mosquitoes. This occurs through the intrinsic nature of environmental and ecological factors in saltmarshes and can also be impacted by anthropogenic factors. In this thesis, my research is focused on *Aedes camptorhynchus*, the temperate mosquito vector of Ross River virus (RRv); the most prominent and ecologically important vector-borne disease in Australia. Understanding the diversity of saltmarsh habitats and the ecology of saltmarsh mosquitoes within these habitats has potential to improve mosquito vector management and human disease.

I conducted a high-level scoping review of research literature undertaken on saltmarsh mosquito vectors in Australia (Chapter 2). I found that saltmarsh ecology, in particular the ecology of saltmarsh mosquito vectors, is poorly understood partly due to the expanse and diversity of saltmarsh habitats across the country, and the number of vectors involved in the transmission of disease. I emphasise the utility of ecological health and ecosystem function with respect to human health, vector management and the need for greater knowledge surrounding local saltmarsh ecology and mosquito vector dynamics.

In Chapter 3, I conducted a comparative study between three superficially similar temperate saltmarshes to identify how environmental conditions and aquatic invertebrate assemblage influence mosquito vector abundance. I identified distinct differences in mosquito assemblage between saltmarsh systems and seasons. Environmental variables were dominant in predicting mosquito

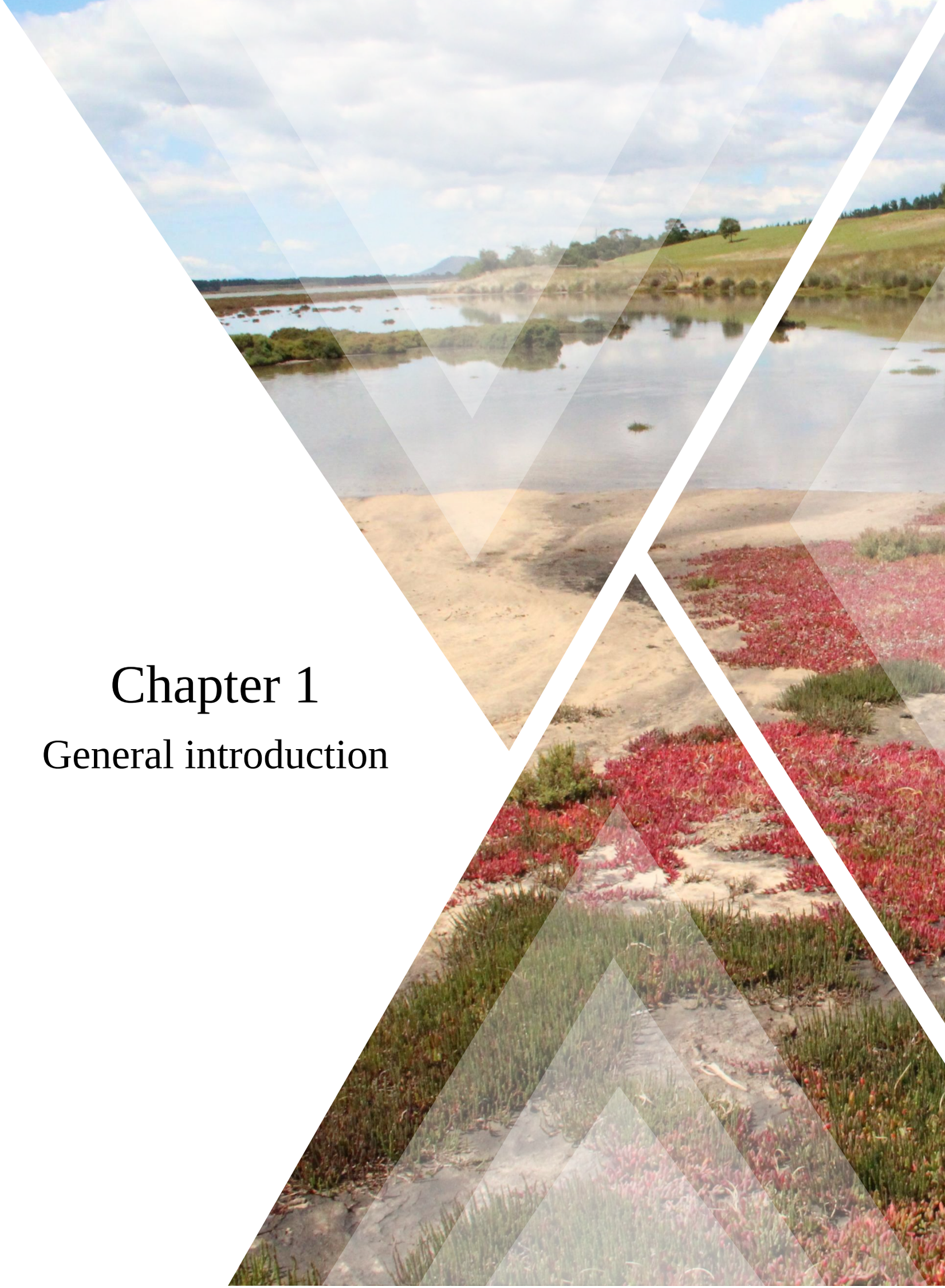
numbers and vegetation (samphire) cover was ubiquitous among saltmarshes in predicting first instar mosquito abundance. Aquatic conditions differed in ability to predict the numbers of *Ae. camptorhynchus* first instars or pupae. The abundance and diversity of aquatic invertebrates contrasted between saltmarsh habitats. Saltmarshes with the least anthropogenic disturbance had greater aquatic invertebrate diversity and fewer vector mosquitoes. This work demonstrates the value in understanding natural saltmarsh aquatic ecology in the context of vector ecology and saltmarsh ecosystem health.

Having identified ecological differences among saltmarsh systems and their influence on vector abundance, I (Chapter 4) conducted a fine-scale field evaluation of the saltmarsh with the highest density of mosquito vectors to determine specific environmental drivers and locations of mosquito egg distribution. I determined that vegetation, particularly samphire (*Sarcornia quinquiflora*), was preferred by *Ae. camptorhynchus* for oviposition relative to shrubby glasswort (*Tecticornia arbuscula*), runnels and bare soil. No correlation between aquatic invertebrates, tidal connectivity, soil moisture and elevation on oviposition habitat selection was found. I discovered that this saltmarsh was less influenced by regular tidal inundations relative to rainfall, resulting in dryer habitat conditions. By understanding oviposition habitat selection and factors determining hatching success we can improve vector surveillance, and management efforts can be more targeted and efficient.

Lastly (Chapter 5), I investigated if a prominent ostracod micro-crustacean affected local populations of *Ae. camptorhynchus*. My aim was to identify if *Ae. camptorhynchus* competed with this abundant microcrustacean leading to changes in development and survival or if the main limitation was resources. I found that the most limiting factor for *Ae. camptorhynchus* survival and development was resources, rather than competition. When resources were limited it resulted in delayed mosquito development, decreased larval survival and smaller emergent adult mosquitoes. Of these three parameters only adult mosquito size changed when food resources were abundant

and ostracod density increased, resulting in decreased adult mosquito size. I conclude that underlying effects of non-culicid aquatic interactions on mosquito development and survival are context dependent and could have the potential to impact vector-borne disease transmission.

In summary, the work presented in this thesis has contributed to a greater understanding of the ecology of mosquitoes in saltmarshes. This research demonstrates the complexity of superficially similar saltmarsh systems and how detailed knowledge of these systems can inform vector surveillance and management; and how aquatic invertebrate interactions within saltmarsh systems may influence vector mosquito abundance, distribution, and potentially disease transmission. The contributions I have made to temperate saltmarsh habitats and mosquito vector research provide insights into the ecology of a prominent RRv vector and the ecological significance of saltmarsh habitats. Moreover, this thesis contributes to the growing knowledge around ecosystem health, with emphasis on the requirement to understand the ecology of vectors in saltmarsh habitats and the potential impact manipulation of these habitats can have to human health through influence on vectorial abundance and life history.



Chapter 1

General introduction

Chapter 1 – General introduction

1.0 Saltmarsh ecology and mosquito vectors

Globally, there are more than 350,000 hectares of saltmarsh ecosystems, excluding regions dominated by mangrove or seagrass (Himes-Cornell et al. 2018). These habitats hold significant ecological value from storage of blue carbon to providing refuge, foraging and breeding grounds for a range of vertebrates (Boorman 1999, Saintilan and Rogers 2013, Rogers et al. 2016), however, they are also important habitats for mosquitoes that transmit pathogens of human health concern (Russell and Dwyer, 2000). Some of the viruses capable of being transmitted by saltmarsh mosquitoes include Japanese encephalitis, Murray Valley encephalitis, Barmah Forest and Ross River viruses (Dale and Knight 2008). Although only the latter two are considered high risk in Australia.

The past decade has seen worldwide increases of vector-borne disease (Gratz 1999, Gubler 2001, Lounibos 2002). Undoubtedly, the expansion of urban development to coastal areas exposes people to greater risk of mosquito contact and disease transmission (Weinstein 1997) but mosquito-borne disease in humans has also been linked with declining ecosystem health (Gratz 1999, Rapport 1999, Cook et al. 2004, Weaver and Reisen 2010, Bader and Williams 2011). Worldwide, saltmarsh habitats continue to be impacted by both direct and non-direct human interference including (but not limited to) habitat modification, chemical applications, nutrification, livestock grazing and the spread of invasive weeds (Adam 2002, Prahalad and Pearson 2013). As a result, the health of these ecosystems is declining with many plant and

animal species now threatened. Yet, research examining the underlying mechanisms driving the spatial differences in vector abundance within saltmarsh systems is rare, and ecological processes within these habitats are poorly understood.

A major factor for the paucity of research on the ecology of saltmarsh systems and vector abundance is the spatial heterogeneity and complexity of these systems (Saintilan and Rogers 2013). It is generally understood that environmental aspects such as geographic location, climate, and hydrological connections contribute to the diversity of vegetation and fauna (Laegdsgaard 2006, McCall and Pennings 2012, Saintilan and Rogers 2013) Geographic location also contributes to diversity with an increase in species richness in cooler climates (Adam 1990, 2002, Saintilan 2009, Zedler 2010, McCall and Pennings 2012). Changes in saltmarsh habitats are particularly evident in Australia, where coastal saltmarshes occupy more than 9,000 km^2 of the coastline, ranging from temperate to tropical climatic zones (Rogers et al. 2016). Across this range the most obvious change is differences in vegetation, with the absence of mangroves in the south of the continent, and this is accompanied by many other ecological differences, including vector mosquito distribution and associated disease incidences (Russell and Dwyer, 2000)

1.1 Australian saltmarsh mosquitoes and vector-borne disease

In terms of incidence, the two most important vector-borne pathogens transmitted by Australian saltmarsh vectors are Ross River virus (RRv) and Barmah Forest virus (BFv). Nationally, these account for more than 5,000 infections per year and cost the Australian economy in excess of AU$10 million/year (Harley et al. 2001). Common symptoms of these diseases include severe joint pain, malaise, fatigue and rash (Doherty et al. 1972, Harley et al. 2001). Transmission naturally occurs between mosquito vectors and reservoir hosts, principally macropod

marsupials (Russell 2002), although other vertebrates may be involved (Doherty et al. 1972, Harley et al. 2001, Stephenson et al. 2018). Humans also appear to have a role as amplification hosts, but do not support the long-term persistence of the virus (Aaskov et al. 1981, Tesh et al. 1981).

Throughout Australia there are several mosquitoes capable of transmitting both RRv and BFv and potentially amplify transmission in peak seasons. Some of the freshwater species include *Culex annulirostris* (Skuse), *Cx. australicus* (Dobrotworsky and Drummond), *Cx. quinquefasciatus* (Say) and *Ae. notoscriptus* (Skuse) (Russell 1998, Harley et al. 2000, Willaims and Rau 2010). Although the most important vectors are the saltmarsh mosquitoes (Table 1.1) with *Aedes vigilax* (Skuse) and *Ae. camptorhynchus* (Thomson) being the two most prominent saltmarsh vectors responsible for most disease transmission events (Ballard and Marshall 1986, Vale et al. 1992, Harley et al. 2001, Kay and Jennings 2002, Russell 2002, van den Hurk et al. 2010). Distribution of these species range from coastal saltmarshes and mangroves to inland brackish and saline waterbodies within the wheatbelt of Western Australia. *Aedes camptorhynchus* is most common across southern Australia and is replaced by *Ae. vigilax* north of its range (Kokkinn et al. 2009).

Table 1.1: The distribution, pathogen transmission, habitat and biology, and public health importance of key saltmarsh mosquitoes in Australia.

Mosquito species[a]	Distribution[b]	Associated pathogens[c]	Habitat and biology[d]	Public health importance
Aedes alternans	SA, Vic., NSW, Qld, NT, WA	RRv	SW/FW; found in coastal saltmarshes and inland areas. Capable of dispersing widely. The larvae are predatory.	Potential nuisance-biting pest active day and night. Vector status is poor
Aedes camptorhynchus	Tas., SA, Vic., NSW, WA (Southern)	BFv, RRv, MVE	SW/B; commonly found in coastal saltmarshes, inland saline regions and grasslands. Larvae have extreme salt tolerances and can tolerate a range of aquatic temperatures. They can disperse several kilometres from their habitat.	Severe nuisance biting pest, with crepuscular activity. One of the most important vectors of RRv and BFv in southern Australia
Aedes vigilax	SA, Vic., NSW, Qld, NT, WA	BFv, RRv, MVE	SW/B; common in coastal saltmarshes and mangroves but can be found in flooded sedgelands. Exceptional dispersal capabilities and is autogenous.	Severe nuisance biting pest day and night. One of the most important pest species in coastal regions of NSW, Qld, NT and WA
Verrallina funerea	NSW, Qld, NT	RRv, BFv	SW/B; common in coastal saltmarshes and mangrove swamps. Does not disperse	Severe nuisance biting pest and is suggested to be an important amplification vector during outbreaks

[a] Compiled from various sources: (Russel 1998; Hugo et al. 2003; Jeffery et al. 2006; Webb 2013)
[b] States and Territories: NSW = New South Wales; NT = Northern Territory; Qld = Queensland; SA = South Australia; Vic. = Victoria; WA = Western Australia, and Tas. = Tasmania.
[c] Arboviruses; BFv = Barmah Forest virus; RRv = Ross River virus; MVE = Murray Valley encephalitis.
[d] Habitat: SW= salt-water, FW = fresh water, B = brackish.

It is well documented that RRv and BFv outbreaks are seasonal, coinciding with climatic conditions that compliment growth and development of mosquitoes, such as greater than average rainfall events, warmer temperatures and higher than average tides (Kelly-Hope et al. 2004). As such, this information is used to inform public health interventions (Tall et al. 2014). Quite often these predictions are modelled on regional climatic data and National Notifiable Disease Surveillance data (NNDSS 2019). However, these models become intrinsically unreliable given the temporally changing climatic conditions, spatial diversity of saltmarshes and disease epidemiology (Weaver and Reisen 2010, Koolhof et al. 2019).

1.2 The biology of saltmarsh mosquitoes

The lifecycle of saltmarsh mosquitoes is such that they have an obligation to aquatic habitats for the larval stages. Eggs are typically laid on vegetation surrounding waterbodies whereby hatching occurs between 2-3 days after hydrological events, such as rainfall or tidal inundations (Bader and Williams 2011). Both larvae and pupae are dependent on standing water for growth and survival with adults emerging after 12-15 days, depending on temperature (Figure 1.1) Consequently, adult females tend to lay eggs in specific regions of the saltmarsh that maximise offspring hatching (Sinclair 1976, Navarro-Silva et al. 2009) often displaying a preference for saltmarsh vegetation, such as dominant herbs including marine couch (*Sporobolus virginicus*) Kunth, samphire (*Sarcornia quinquiflora*) and seablight (*Sueda australis*) (Kay and Jorgensen 1986, Gislason and Russell 1997, Webb and Russell 1999), located in areas of increased soil moisture and proximity to tidal inundations (Gislason and Russell 1997, Dale et al. 2008).

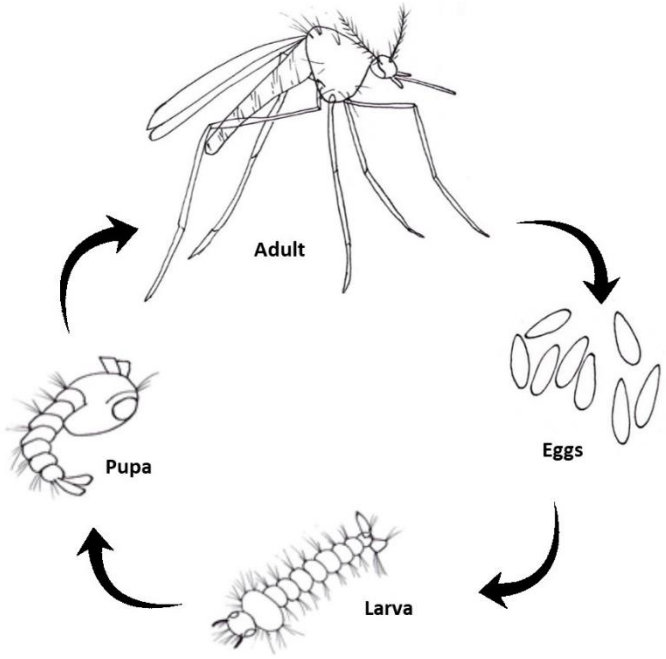


Figure 1.1 Lifecycle of Aedes mosquito displaying egg, larva, pupa and adult stages.

Other factors influencing mosquito oviposition is the presence of antagonistic vertebrates and invertebrates (competitors and predators). Research has demonstrated how both predators and competitors can alter mosquito oviposition (Eitam and Blaustein 2004, Wachira et al. 2010), with others describing the impact these encounters have to larval survival, growth and development (Mokany and Shine 2003, Knight et al. 2004, Stav et al. 2005, Juliano 2009, Vonesh and Blaustein 2010), potentially altering mosquito fitness and vector capacity (Alto et al. 2005, Bara et al. 2015). However, female mosquitoes select oviposition locations according to a range of site-specific cues which can vary between species (Vonesh and Blaustein 2010) and there is little research on the spatial differences between mosquito oviposition sites within saltmarshes.

Saltmarsh mosquitoes also demonstrate many physiological adaptations which enable them to survive in environmental extremes such as hypersaline conditions, excessive temperatures and extremely ephemeral environments (Barton and Aberton 2005, Kokkinn et al. 2009, van Schie

et al. 2009). Regional adaptations to environmental extremes have been shown to occur between species and even within mosquito species (van Schie 2006). With the diversity and ephemeral nature of saltmarsh systems, determining local outbreaks of mosquitoes important to human disease encompasses many environmental and ecological aspects (Carver et al. 2010) and the degree and magnitude of such are not only species specific but also vary both regionally and locally (Jacups et al. 2008). One historic example of exceptional local knowledge, surveillance and vector control was the eradication of *Ae. camptorhynchus* in New Zealand during 1998. This program contributed significantly to research on surveillance and management strategies and remains the only program to successfully eradicate an invasive mosquito in a country/region (Kay and Russell 2013). Thus, demonstrating that by understanding the spatial distribution and abundance of mosquitoes within the saltmarsh, opportunities exist in improved control and management of mosquito populations as well as minimising human disease.

1.3 Research applications

Interest in saltmarsh habitats has steadily increased owing to the encroachment of urban development and elevated risk of mosquito transmitted disease (Lyth et al. 2005). Expanding our understanding of saltmarsh ecology, the diversity of these habitats and the species that utilise these environments will not only improve the perceived value of these systems but benefit restorative efforts. Of importance are the saltmarsh mosquitoes in these habitats that vector human disease. Early research was largely focused on improved methods of vector control (Dale and Knight, 2012), with generalisations of the adverse impacts of chemical control to other aquatic invertebrates through laboratory tests or indicator species (Brown et al. 1999). Furthermore, models informing early warning systems have been calculated by climatic

models and abundance of vectors obtained from adult traps (Jacups et al. 2015, Jacups et al. 2008b), with little regard to the diversity and influence potential aquatic fauna could have towards mosquito intervention. With improved knowledge of the aquatic ecology in saltmarsh habitats and the comparative differences among saltmarsh systems we can better understand mosquito productivity and disease and provide mechanisms that will improve mosquito management and thus human health.

1.4 Thesis objectives and structure

The purpose of this thesis is to investigate the ecology of a saltmarsh vector in Tasmania, *Ae. camptorhynchus* (Figure 1.2), and to better understand environmental features, in temperate landscapes, that determine mosquito populations, abundance and survival. To address these objectives, I present four core chapters (Figure 1.3), in the form of independent publishable units, bound by this general introduction and a general discussion (Chapter 6). I outline each of these chapters below.

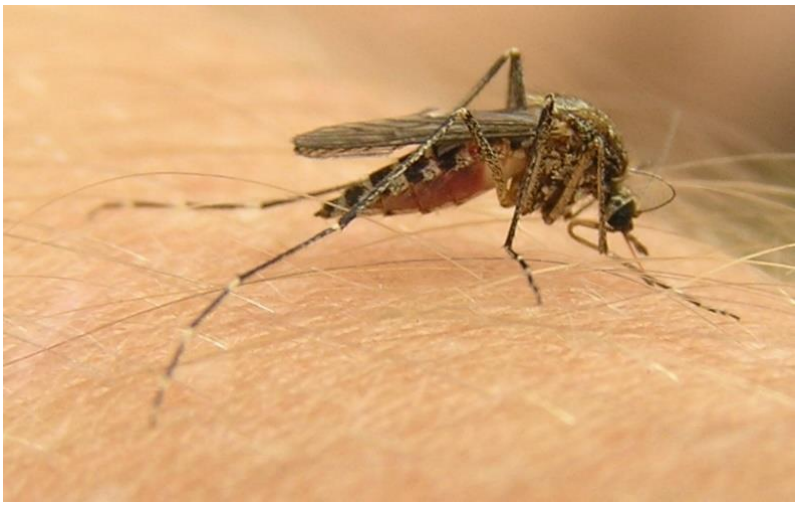


Figure 1.2: *Aedes camptorhynchus,* a focal mosquito of this PhD thesis. Photo courtesy of Scott Carver.

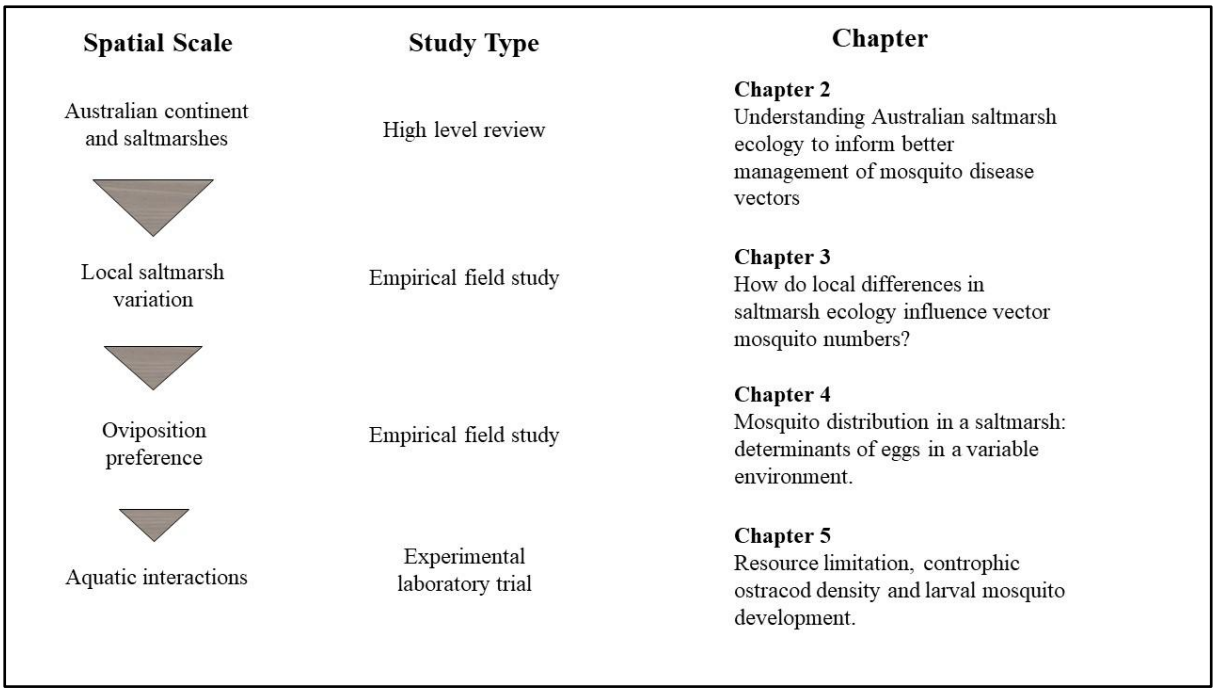


Figure 1.3: Schematic overview of thesis structure.

Chapter 2 addresses the history and research themes of saltmarsh vector-borne disease research in Australia and has been submitted to EcoHealth for publication. The paper focuses on mosquitoes that vector Ross River virus – Australia's most prominent vector-borne disease. While this research has historically been related to mosquito control (Dale and Knight 2012), there is yet to be a comprehensive synthesis of what has been done particularly identifying current research gaps surrounding saltmarsh vectors. Hence, in this chapter, I present a high-level review on the research themes associated with saltmarsh vectors in Australia and highlight important future areas for saltmarsh mosquito research. This provides important context to the subsequent chapters of this thesis.

Saltmarsh habitats are commonly distinguished by their similarity in vegetative floral structure and composition (Saintilan 2009); however, these habitats can be distinctively different. Understanding the factors in which these habitats differ provides valuable insight to studies of

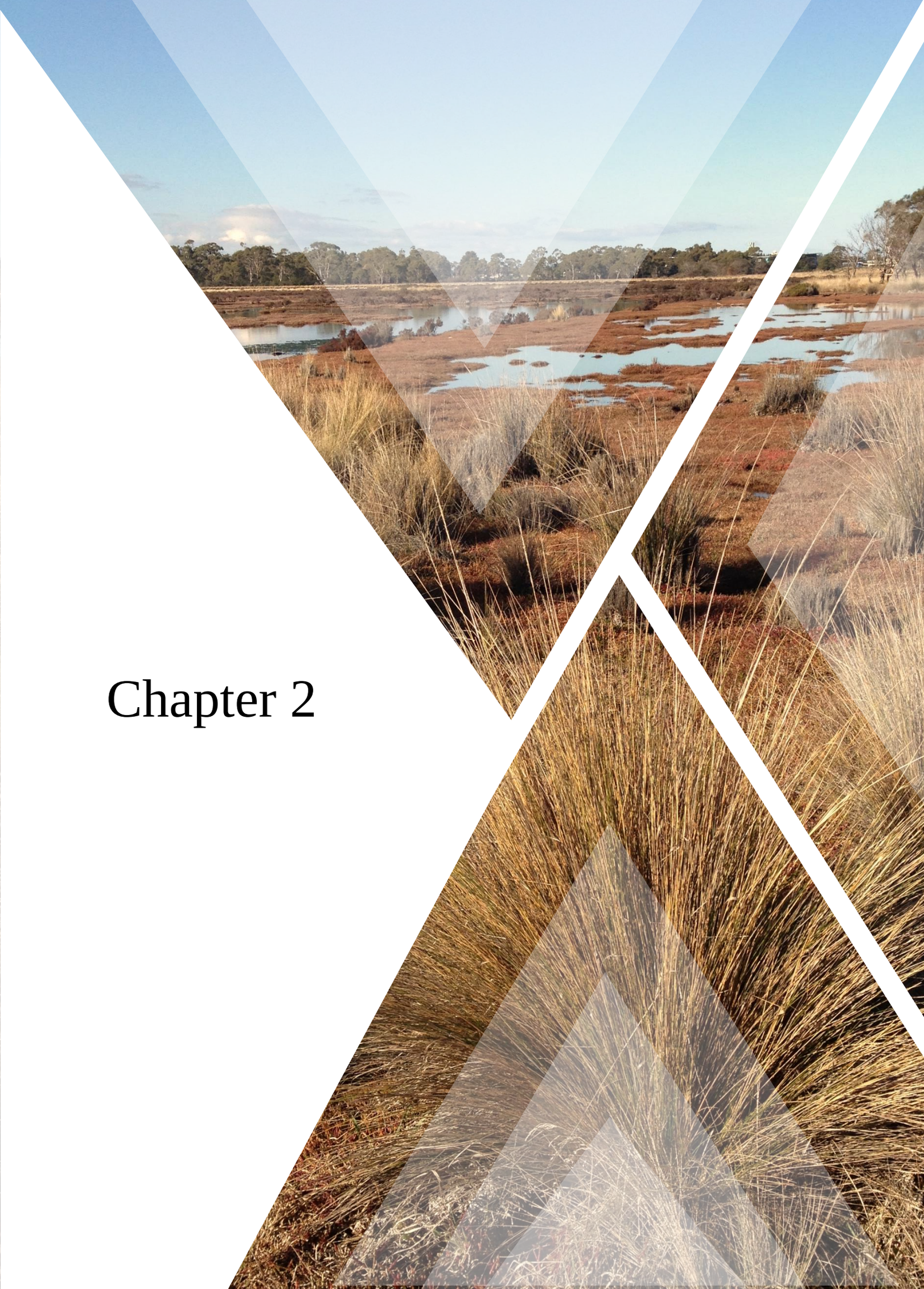
vector dynamics. In Chapter 3, I present detailed investigations into three local saltmarsh habitats, each with distinct historical anthropogenic influences, which has been published in Medical and Veterinary Entomology (Rowbottom et al. 2020). The alteration of saltmarsh habitats for development and mosquito control have been common practice all over the globe (Gedan et al. 2009, Rochlin et al. 2012). However, an often-neglected aspect of saltmarsh mosquito management is how ecological health (often disturbed through these processes) can influence the balance of pest species (Corvalán et al. 2005). Thus, this chapter provides valuable insight to the benefits of understanding saltmarsh ecology for mosquito management.

In Chapter 4, I present environmental factors that determine the spatial distribution of *Ae. camptorhynchus* within temperate saltmarsh habitats, which has been published in the Journal of Vector Ecology (Rowbottom et al. 2017). While oviposition preferences are known for other vectors and regions, oviposition by temperate mosquitoes is relatively unknown. Through this research I identified regions of the saltmarsh that are key features for mosquito production, allowing me to contextualize the landscape characteristics associated with increased mosquito activity. These results contribute to the body of literature surrounding vector distribution and provide key information for predicting potential vector habitat which will contribute to future management of *Ae. camptorhynchus* in southern temperate regions.

Understanding the physiological consequences of biotic interactions on mosquito vectors can reveal compounding factors that lead to changes in mosquito development or mortality which provides the potential for management practices. In Chapter 5, I explore the physiological and developmental impacts of a putative aquatic competitor on *Ae. camptorhynchus* which has been published in *PLoS one* (Rowbottom et al. 2015). I reveal that recognising the fundamental/inherent ecology of mosquitoes and that of other biotic organisms within these habitats could provide considerable improvements towards understanding disease dynamics and management.

Finally, in Chapter 6, I synthesise my findings and discuss the broader contribution of this thesis to our understanding of saltmarsh vector ecology within Australia. I discuss management strategies for *Ae. camptorhynchus,* and for the broader management of vector mosquitoes in saltmarshes. I also propose several directions for future research that I have highlighted through my work.

The three saltmarsh habitats which were used for research in this thesis can be viewed in Appendix I.



Chapter 2

Chapter 2 – Understanding Australian saltmarsh ecology to inform better management of mosquito disease vectors

Raylea Rowbottom [a], Scott Carver [b], Leon A Barmuta [b], Philip Weinstein [c], Geoff R Allen [a]

[a] Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Australia

[b] School of Natural Sciences (Biological Sciences), University of Tasmania, Hobart, Australia

[c] School of Public Health, University of Adelaide, Adelaide, Australia

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Author contributions: SC, RR, GA conceived and designed the research. RR conducted the literature search, conceptual model and selection criteria. RR analysed and reviewed the material. RR wrote the manuscript with critical feedback from all authors.

2.0 Abstract

Australian saltmarshes are important habitats for mosquito vectors of disease. Synthesising research themes surrounding vector ecology within saltmarsh habitats can inform research directions and improved mosquito management practices. There were 50 relevant peer-reviewed publications between 1970 and 2019 identified for review. Studies were classified into four broad topics: distribution and abundance (n=29), management and control (n=15), biology/physiology (n=5) and ecological interactions (n=1), with most studies (66%) from Queensland and New South Wales. Temporal trends in research foci included moving from vector management and control by habitat modification and insecticides (pre- 1980s), to vector distribution and abundance (1980s-90s) and back to vector control, but by saltmarsh restoration and vector biology/ecology (late 1990s onwards). The most common suggested topic of future research was improved surveillance measures, vector control and saltmarsh hydrology. Knowledge gaps identified in the literature included comprehensive spatial mapping of mosquito vector abundance and distribution, detailed understanding of local saltmarsh hydrology, environmental influences on vector mosquito biology, and the regulatory effects of aquatic ecological interactions to vector populations. With a growing demand for less conventional, chemical control measures, more research is required on saltmarsh ecology and aquatic interactions that may prove effective in managing mosquito vectors, and benefit human health.

Keywords

Saltmarsh, ecology, ecosystem health, human health, vectors, mosquito, RRv, BFv

2.1 Introduction

Healthy, biodiverse, well-functioning ecosystems provide services to human populations - services that sustain healthy human populations, and come in the form of provisions (food, water, fibre), regulation (climate control, disease regulation), and culture (sense of place, recreation) (Corvalán et al. 2005). When ecosystems are disrupted, as a result of human activity, ecosystem services are also disrupted. For example, allowing stock to graze in water catchments contaminates the water supply; clearing forests contributes to global warming; and urbanisation leads to a loss of contact with nature. In coastal saltmarshes, suburban encroachment, drainage, eutrophication, pollution, hydrological and climatic changes all have the potential to damage the integrity and functionality of these fragile ecosystems. It is now well-established that such ecosystem disruptions can lead to loss of biodiversity and disease regulation (Ostfeld and Keesing 2000, Corvalán et al. 2005), often because of an increase in disease vectors that have been released from the pressures of competition and predation (O'Sullivan et al. 2008). Carver et al. (2011) and Rowbottom et al. (2020) showed that this was likely the case in Tasmanian saltmarshes, where species diversity declined with increasing abundance of the Ross River virus vector, *Aedes camptorhynchus* (Thomson), as well as more human infections with the virus. With a growing environmental consciousness and in the consideration of alternatives to mosquito control by habitat destruction or insecticides, we felt it timely to review the vector ecology of mosquitoes in saltmarshes with the aims of (1) providing an evidence base and reference resource for saltmarsh mosquito research within Australia, and (2) identifying gaps in knowledge and targets for future research.

Saltmarshes are arguably the most important habitat for mosquito borne disease in Australia (Russell 1995). The two most prominent Australian arboviruses, Ross River virus (RRv) and Barmah Forest virus (BFv) are largely vectored by the saltmarsh mosquitoes, *Aedes vigilax* (Skuse) and *Ae. camptorhynchus*, although freshwater and brackish water vectors can also be

involved (e.g. *Culex annulirostris, Ae. notoscriptus, Ae. normanensis, Ae. procax* and *Verrallina. funerus*) (Russell 1995). Together, notifications of these viruses have reached approximately 115,000 over the past 20 years (NNDSS 2019) and more than a decade ago were estimated to cost approximately AU$10-15 million/year (Woodruff and Bambrik 2008). Saltmarsh habitats hosting RRv vectors cover approximately 13,000 km^2 of the Australian coastline (Rogers et al. 2016). Most saltmarshes are coastal, whereas others can extend inland, and these habitats vary substantially in flora, fauna, climatic conditions, tidal influences and other factors (Saintilan 2009). For example, saltmarsh habitats in the southern parts of the continent are dominated by prostrate herbaceous grasses and succulent shrubs, whereas northern saltmarshes are often fringed by mangroves (Saintilan and Rogers 2013). Because of their variable conditions, the diversity and abundance of mosquito assemblages in saltmarshes across Australia also varies both in space and time. This variation creates challenges for the management of saltmarsh vector mosquito populations.

Saltmarsh vector research in Australia dates to the early 1970s, covering multiple disciplines and phases of RRv activity (Table 2.1). Notifications of this disease have exhibited a general increase over time (Figure 2.1), and in recent years the focus of mosquito vector research has shifted from habitat modification and insecticide control to conservation and restoration of these habitats to improve ecosystem function and biodiversity (Brady et al. 2002, Dale and Knight 2012). Collectively this has highlighted significant knowledge gaps, especially the benefits provided by natural saltmarsh ecosystems to society and mosquito vector management (Prahalad 2019). It is important to acknowledge that there have been other reviews undertaken in related fields (Supplementary I), however, there has yet to be any synthesis of mosquito vector research associated with saltmarsh habitats in Australia. Accordingly, we posed the following key questions (1) What have been the research themes surrounding mosquitoes in Australian saltmarshes to date? (2) Where are the critical research gaps for saltmarsh vector

mosquito research in Australia? And (3) what are key future research directions? To achieve this, we (i) first systematically quantified and summarised the literature on saltmarsh mosquito research in Australia and then (ii) provided a narrative discussing the major research themes contextualising the gaps and future directions.

Table 2.1: List of broad research themes associated with peer reviewed published papers from the final selection of articles based on the selection criteria on mosquitoes in Australian saltmarshes since 1970. Developmental stages include E (egg), L (larval), and A (adult) or a combination of each.

Topic	Date range	State (# articles)	Publications by developmental stage					Key focus within major topics
			E	L	A	E/L	L/A	
Distribution & Abundance	1992-2018	Qld (10)	4	2	2	1	1	Seasonal trapping, environmental factors, habitat proximity (urban vs saltmarsh), vector diversity and abundance.
		NSW (7)	4		2		1	
		Vic (1)			1			
		SA (5)			3		2	
		WA (2)			2			
		NT (2)			2			
		Tas (2)	1	1				
Management & Control	1973-2012	Qld (11)		11				Chemical, surveillance, saltmarsh modifications
		NSW (2)	1	1				
		NT (2)		1	1			
Biology/physiology	1996-2010	Qld (3)		1	2			Salinity tolerance, egg longevity, vector competence, and autogeny
		SA (1)	1					
		WA (1)		1				
Ecological interactions	2015	Tas (1)		1				Resource and competition

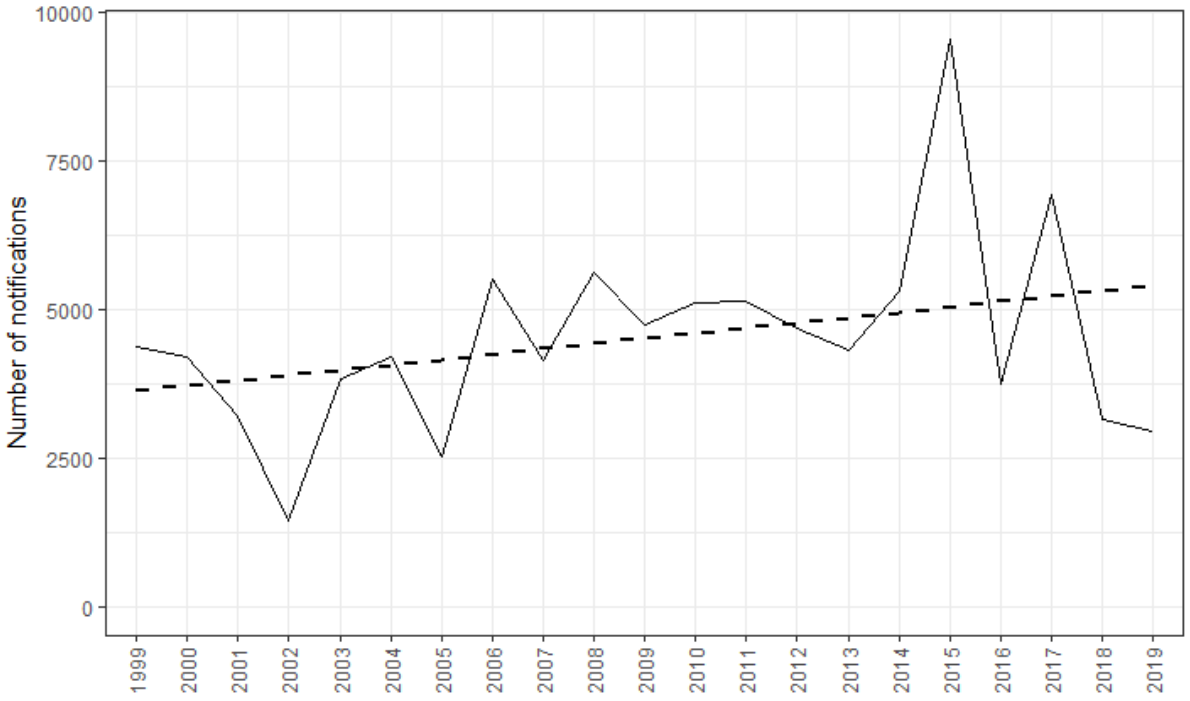

Figure 2.1: Number of notified cases of RRv in Australia by year from 1999–2019, with linear regression trendline (NNDSS 2019).

2.2 Methods

We conducted a systematic literature search to identify the research themes that have been conducted on mosquitoes in Australian saltmarshes, particularly on mosquito vectors of human disease. First, we searched the title, topic, keywords and abstracts within electronic databases (Web of Science, PubMed and Scopus) for articles published between 1970 and 2019 using the following search criteria: (saltmar* OR "salt mar*") AND (Aust* OR "New South Wales" OR Tasmania* OR Queensland OR "Northern Territory" OR "West* Austral*" OR Vic* OR "South Austral*") AND (mosq*). The asterisk (*) operator was used as a wildcard to search for all possible variations of the word. Following the systematic literature review method outlined by Pickering et al. (2015), only original works on saltmarsh mosquitoes within

Australia were included; review papers, reports of freshwater culicid species and articles exclusively on mangrove habitats were excluded as these are entirely different systems. From selected articles, the bibliographies were searched for further references. The selection criteria used to include articles are provided (Figure 2.2), along with a complete list of publications used in this scoping review (Supplementary II). For each article, we recorded the following information: authors, primary author, year of publication, location of study, disease studied, mosquito species studied, laboratory/field/combination study, type of study (vector distribution and abundance, management and control, epidemiology, biology and ecology), variables assessed (e.g. for control studies: chemicals used, application, mosquito response (increase, decrease, neutral), non-target assessment, post application analysis), key results and future directions. Given that the epidemiology of vector-borne disease has recently been reviewed (Stephenson et al. 2018), and that epidemiological research generally did not directly relate to saltmarshes, we removed articles under the topic "epidemiology".

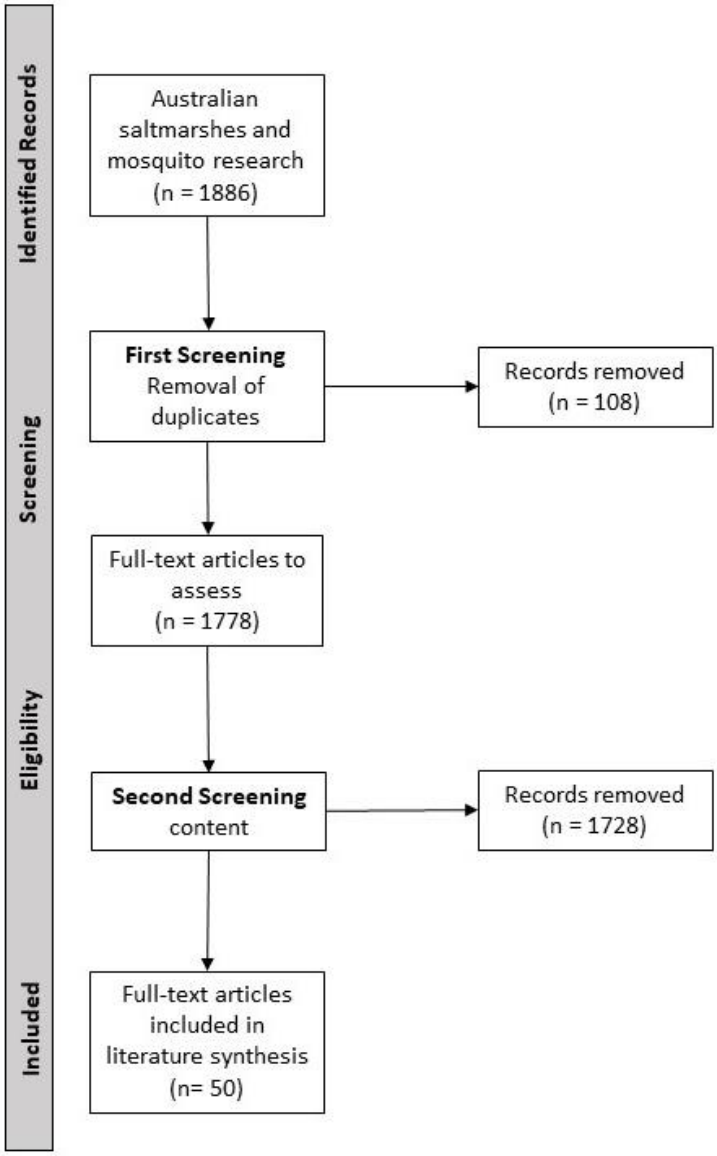

Figure 2.2: Methodology and search criteria used in the scoping literature review following review methods by Pickering et al. (2015)

2.3 Results

Fifty original articles met our selection criteria. Of these, one investigated the ecology of a saltmarsh mosquito, five researched aspects of vector biology or physiology, 15 focused on vector management within saltmarshes and 29 described vector abundance and distribution (Table 2.1). The largest proportion of studies (48%) was from Queensland, followed by New South Wales (18%), and South Australia (12%), with Northern Territory, Tasmania, Western Australia, and Victoria making up the remaining 22% (8%, 6%, 6% and 2% respectively).

Prior to 1980 the focus of mosquito research was management and control, especially using insecticides (Table 2.1). From early 1980 through to the early 21st century research on mosquito distribution and abundance was ten times greater than any other topic and was the largest field of research spanning 32 years. Towards the end of the 1990s, the research focus shifted back to management and control, but chiefly by saltmarsh restoration. Of the five studies focusing on mosquito biology/physiology, each concentrated on different aspects of biology; these were published between 1986 and 2011 with each publication being separated by several years. The field least covered was ecological interactions, with only one study based in Tasmania.

Approximately equal effort was expended on adult (30%) and larval (38%) research, with adult research focussed in the topic distribution and abundance (80%) while larval research was mostly management and control topics (68%). Research on mosquito eggs comprised 22% of the literature (81% of which concerned distribution and abundance), while the remaining 10% of studies investigated more than one stage of mosquito life history.

We summarised the future directions proposed across the reviewed articles and identified seven themes: aquatic ecology, control, epidemiology, hydrology, local environment, surveillance, and vector ecology (Table 2.2). Of these categories, the most prominent topic for future research commonly identified was improved surveillance (31%), with vector ecology and

control equally recommended (18%). Improved understanding of local environment and hydrology were equally suggested within the literature (12% and 13% respectively). The area of research least mentioned was aquatic interactions and epidemiology (both 4%).

Table 2.2: Summary of future direction themes suggested from articles across the four study topics.

Future Direction	Study Topic				Total Future Direction
	Distribution & Abundance	Management & Control	Biology/physiology	Ecological interactions	
1. Aquatic interactions	2	-	-	1	3
2. Control[ɟ]	6	6	-	-	12
3. Epidemiology	2	-	1	-	3
4. Hydrology[℗]	2	7	-	-	9
5. Local environment	5	1	2	-	8
6. Surveillance[*]	16	4	1	-	21
7. Vector ecology	9	1	2	-	12

[ɟ] Control includes; timing, location and chemical selectivity.

[℗]Hydrology includes; saltmarsh rehabilitation, saltmarsh modification, understanding local hydrology

∗surveillance includes; design, methods, risk assessment/prediction and collaboration with other technologies such as GIS and Remote sensing.

2.4 Discussion

The discussion is focused on the categorised themes of current research surrounding saltmarsh mosquito vectors, with the aim to emphasise gaps in knowledge surrounding saltmarsh systems and required future research. It is important to emphasise that although mangrove systems were excluded from this review, these habitats are important for mosquito vectors and there is a wealth of knowledge published that has applications for the future development of public health assessment, disease risk and implementation of mosquito vector management.

2.4.1 Theme 1: Distribution and abundance

Surveillance of vector distribution and abundance is a vital component for understanding the potential threats of disease. Evaluation of saltmarsh vegetation and habitat structure suitable for oviposition and mosquito distribution has improved management of vectors through more targeted control and provided information of current and potential habitats for vector distribution (Dale et al. 2008, Jacups et al. 2009, Jacups et al. 2012, Rowbottom et al. 2017). Regular surveillance of mosquito populations provides information on local seasonal abundance and status of vectors (Turner 2002, Jacups et al. 2008b) and predictive models have been developed for mosquito vectors through research on environmental drivers (Woodruff et al. 2003, Yang et al. 2009, Jacups et al. 2015). However, continual assessment of the effectiveness of surveillance methods and control is also important.

Given the biology of mosquitoes, there is an intimate relationship with habitat hydrology and aquatic conditions (Webb and Russell 1999, Jacups et al. 2012, Jacups et al. 2015). Hydrological conditions of coastal saltmarshes differ from those in more estuarine situations (Adam 2002) and many aquatic conditions affect mosquito species differently (Russell et al. 1992, de Little et al. 2009, Kokkinn et al. 2009, Carver et al. 2011) changing both the spatial distribution and abundance of vectors (Miller et al. 2005, Williams et al. 2012, Johnston et al.

2014, Walker et al. 2018). While reassessing surveillance techniques and methods for regions is useful, many of these techniques are both time consuming and costly. With advances in technology, we now have the capability of combining traditional surveillance methods with mapping large areas remotely through geographic information systems and remote sensing (Dale et al. 1998, Walker et al. 2018). However, there is little mention of additional costs involved with these methods and for these to be effective clear management objectives and direction for the future of vector surveillance is required.

To date, much of the surveillance within Australia has been focussed on improved understanding, prediction and management of nuisance vectors and outbreaks of disease. While much surveillance in Australia has been initiated in a reactional manner, it has provided direction for future research with gaps evident in vector/reservoir host systems, longer seasonal data, and site-specific information on hydrology, vegetation, species composition and ecology. With the changing focus on vector control and saltmarsh management, consideration of the fit of current surveillance is required to continue to effectively move forward. For example, if the focus is saltmarsh restoration, better understanding of saltmarsh ecology and the local aquatic invertebrate communities that can influence mosquito populations is necessary (Carver et al. 2011, Jacups et al. 2012). For regions with highly dynamic seasonal and yearly mosquito numbers and variable outbreak seasons, investment in topographical models focusing on hydrology may be worthwhile, although in some regions where tide predictability is low (Rowbottom et al. 2020) further assessment of the habitat may be necessary. Overall, for future surveillance of vectors to be cost effective and efficient, the objectives need to be clear and commensurate with intended outcomes and applications of the data acquired.

2.4.2 Theme 2: Management and control

Over the past two decades, more than 60% of RRv outbreaks occurred in Queensland (37%), New South Wales (14%) and Western Australia (13%) (NNDSS 2019), and hence management and control research has been focused in these regions. Furthermore, given the absence of *Ae. camptorhynchus* outside of southern temperate regions, the primary mosquito studied has been *Ae. vigilax.*

Pre 1980, the form of control utilised was chemical control, mainly in the form of larvicides typically the organophosphate larvicide "Abate" active ingredient temephos (Kay et al. 1973). Beyond 1980 synthetic compounds were tested for control including further organophosphates but also insect growth regulators (pyriproxyfens and s-methoprene) (Brown et al. 1999, Webb et al. 2012), and more selective mosquito control larvicides emerging such as *Bacillus thuringiensis israelensis* (Brown et al. 1998, Brown et al. 2001). It was also during this time period that the approach to control began to focus on implementing passive methods through the restoration of saltmarsh hydrology. Some of the methods utilised have been runnelling (Dale et al. 1993), grid ditching and open marsh water management (Dale et al. 2002), however, in some circumstances, the environmental impact of these techniques outweighed the impact on control (Saffigna and Dale 1999, Turner and Streever 1999).

The top priorities to emerge for future research among management and control articles were a better understanding of saltmarsh hydrology (37%) and improved vector control (32%), with more than half of the future research outcomes mentioning these topics. The next most mentioned field of future research was better surveillance techniques. Through improved understanding of both mosquito distribution within a saltmarsh and abundance over time, future control measures can be optimised, thereby reducing cost, time and improving efficiency.

2.4.3 Theme 3: Biology/physiology

There are many aspects of mosquito biology that can influence its ability to vector diseases. In the case of saltmarsh mosquitoes, the ability to develop and survive in highly changeable conditions is impressive. From the five identified studies on saltmarsh vector biology, all researched different aspects of saltmarsh vector biology/physiology, with most studies driven by species invasions or disease outbreaks/risk.

Recent research has begun to uncover intrinsic vector associations within saline aquatic habitats and saltmarshes (van Schie et al. 2009, Bader and Williams 2011). From this research, we have a better understanding of how these prominent vectors can withstand the harsh environmental conditions often present in these environments. For example, a laboratory study driven by an eradication program in New Zealand, demonstrated the ability of *Ae. camptorhynchus* eggs to remain viable for 15 months without water (Bader and Williams 2011). Furthermore, with increasing inland dryland salinity research has discovered the range of salinity tolerances is both vast and varied between inland (8-30 ppt.) and coastal (>50 ppt.) *Ae. camptorhynchus* populations (van Schie et al. 2009). However, what remains unclear from this research is how the local environment and the presence of other fauna affect mosquito biology and physiology (Table 2.2). Hugo et al. (2003) has provided some evidence, through description of changes in autogeny rates of *Ae. vigilax* between two separate saltmarsh habitats with laboratory tests proving that vector diet and body size influence autogeny and fecundity. Given that research by Hugo et al. (2003) and altered vector development in the presence of other aquatic fauna (Rowbottom et al. 2015), there is strong reason to contextualise studies of vector biology/physiology with relevant conditions vectors experience in nature (see *Theme* 4).

2.4.4 Theme 4: Aquatic ecology

The relevance of the aquatic ecosystem for vector mosquitoes in Australia is vastly under researched, particularly given the range of habitats, climatic conditions and biotic interactions that are potentially occurring. In Australia, only two states, Tasmania and Western Australia, were represented in which environmental variability and vector abundance amongst saltmarsh habitats have been being investigated. From this research, we can begin to understand how mosquito assemblages within saltmarsh habitats change with surrounding land use (Carver et al. 2011), extremes in environmental conditions, changes in aquatic fauna (Carver et al. 2009, Rowbottom et al. 2020) and how differences between habitat conditions influence aquatic fauna and in turn mosquito assemblages (Rowbottom et al. 2020). Greater in-depth understanding of these habitats, their aquatic assemblages and hence ecological interactions within have the potential for improved vector management.

2.5 Conclusions

The overall emphasis is that prominent saltmarsh vectors important to human disease in Australia are highly versatile, competent vector mosquitoes, capable of adapting to a broad range of environmental conditions. With such adaptability and the wide distribution of mosquito vectors across a range of climatic regions in Australia, there is a need for greater research on individual populations of vector mosquitoes in their local conditions and how this varies spatially. Few studies have established clear ecological drivers of mosquito abundance within a saltmarsh, and such an understanding could be a critical consideration of limited or non-insecticidal control of vector breeding. It remains unclear, for example, why some regions are more prone to mass abundance of mosquitoes than others; and by enhancing such understanding it may be highly beneficial for the effective control of mosquito vectors to

protect public health. Effective control will require better long-term hydrological data, vegetation mapping, aquatic invertebrate diversity surveys, and an assessment of how changes to climate may alter the dynamics of these habitats in the future. To help researchers and public health practitioners move in that direction, we hope that this review will provide a valuable synthesis of the data available, as well as guide research areas for the future.

Supplementary Material – Chapter 2

I: List of review themes related to Ross River virus research and the associated references.

Topic	References
RRv epidemiology and ecology	(Weinstein 1997, Harley et al. 2001, Russell 2002, Jacups et al. 2008a, Aaskov et al. 2012, Tall et al. 2014, Liu et al. 2017, Stephenson et al. 2018)
Climate change and vector-borne disease	(Aaskov et al. 1985, Tong et al. 2002, Zhang et al. 2008, McMichael and Lindgren 2011, Yu et al. 2014, Lyth and Holbrook 2015)
Vector-borne disease in urban areas	(Dale and Knight 2008, Eder et al. 2018)
Surveillance and control	(Russell and Kay 2008, van den Hurk et al. 2012)
Coastal habitats, their vulnerability and adaption	(Adam 2002, Saintilan and Rogers 2013, Leo et al. 2019)

II: List of research articles used in this high-level review.

Bader CA, Williams CR 2011. Eggs of the Australian saltmarsh mosquito, *Aedes camptorhynchus*, survive for long periods and hatch in instalments: implications for biosecurity in New Zealand. *Medical and Veterinary Entomology* 25:70-76

Barton PS, Aberton JG, Kay BH 2004. Spatial and temporal definition of *Ochlerotatus camptorhynchus* (Thomson) (Diptera: Culicidae) in the Gippsland Lakes system of eastern Victoria. *Australian Journal of Entomology* 43:16-22

Brown MD, Carter J, Watson TM, Thomas P, Santaguliana G, Purdie DM, Kay BH 2001. Evaluation of liquid *Bacillus thuringiensis var. israelensis* products for control of Australian Aedes arbovirus vectors. *Journal of the American Mosquito Control Association* 17:8-12

Brown MD, Thomas D, Mason P, Greenwood JG, Kay BH 1999. Laboratory and field evaluation of the efficacy of four insecticides for *Aedes vigilax* (Diptera: Culicidae) and toxicity to the nontarget shrimp *Leander tenuicornis* (Decapoda: Palaemonidae). *Journal of Economic Entomology* 92:1045-1051

Brown MD, Thomas D, Watson K, Kay BH 1998. Laboratory and field evaluation of efficacy of VectoBac 12AS against *Culex sitiens* (Diptera: Culicidae) larvae. *Journal of the American Mosquito Control Association* 14:183-5

Carver S, Goater S, Allen GR, Parr R, Fearnley E, Weinstein P 2011. Relationships of the Ross River virus (Togoviridae: *Alphavirus*) vector, *Aedes camptorhynchus* (Thomson) (Diptera: Culicidae), to biotic and abiotic factors in saltmarshes of south-eastern Tasmania, Australia: a preliminary study. *Australian Journal of Entomology* 50:344-355

Chapman HF, Hughes JM, Jennings C, Kay BH, Ritchie SA 1999. Population structure and dispersal of the saltmarsh mosquito *Aedes vigilax* in Queensland, Australia. *Medical and Veterinary Entomology* 13:423-430

Dale P, Knight J, Griffin L 2014. Comparing *Aedes vigilax* eggshell densities in saltmarsh and mangrove systems with implications for management. *Insects* 5:984-990

Dale PE, Ritchie SA, Chapman H, Brown MD 1999. Eggshell sampling: quantitative or qualitative data? *Journal of the American Mosquito Control Association* 15:74-6

Dale PER 2008. Assessing impacts of habitat modification on a subtropical salt marsh: 20 years of monitoring. *Wetlands Ecology and Management* 16:77-87

Dale PER, Chapman H, Brown MD, Ritchie SA, Knight J, Kay BH 2002. Does habitat modification affect oviposition by the saltmarsh mosquito *Ochlerotatus vigilax* (Skuse) (Diptra: Culicidae)? *Australian Journal of Entomology* 41:49-54

Dale PER, Dale PT, Hulsman K, Kay BH 1993. Runnelling to control salt-marsh mosquitoes - long term efficacy and environmental impacts. *Journal of the American Mosquito Control Association* 9:174-181

Dale PER, Hulsman K, Harrison D, Congdon B 1986. Distribution of the immature stages of *Aedes-vigilax* on a coastal salt-marsh in Southeast Queensland. *Australian Journal of Ecology* 11:269-278

Dale PER, Knight J, Kay BH, Chapman H, Ritchie SA, Brown MD 2008. Habitat characteristics and eggshell distribution of the salt marsh mosquito, *Aedes vigilax*, in marshes in subtropical eastern Australia. *Journal of Insect Science* 8:1-8

Dale PER, Knight JM 2006. Managing salt marshes for mosquito control: impacts of runnelling, open marsh water management and grid-ditching in sub-tropical Australia. *Wetlands Ecology and Management* 14:211-220

Dale PER, Ritchie SA, Territo BM, Morris CD, Muhar A, Kay BH 1998. An overview of remote sensing and GIS for surveillance of mosquito vector habitats and risk assessment. *Journal of Vector Ecology* 23:54-61

Gislason GM, Russell RC 1997. Oviposition sites of the saltmarsh mosquito, *Aedes vigilax* (Skuse) (Diptera: Culicidae), at Homebush Bay, Sydney, NSW - A preliminary investigation. *Australian Journal of Entomology* 36:97-100

Hugo LE, Kay BH, Ryan PA 2003. Autogeny in *Ochlerotatus vigilax* (Diptera: Culicidae) from southeast Queensland, Australia. *Journal of Medical Entomology* 40:897-902

Hulsman K, Dale PE, Kay BH 1989. The runnelling method of habitat modification: an environment-focused tool for salt marsh mosquito management. *Journal of the American Mosquito Control Association* 5:226-34

Jacups S, Warchot A, Whelan P 2012. Anthropogenic ecological change and impacts on mosquito breeding and control strategies in salt-marshes, Northern Territory, Australia. *EcoHealth* 9:183-194

Jacups SP, Carter J, Kurucz N, Mcdonnell J, Whelan PI 2015. Determining meteorological drivers of salt marsh mosquito peaks in tropical northern Australia. *Journal of Vector Ecology* 40:277-281

Janes R, Jardine A, Neville PJ, Nicholson J, Lindsay MDA 2018. Mosquito (Diptera: Culicidae) communities of metropolitan Perth, Western Australia, and implications for managing pest and public health risks. *Austral Entomology* 57:324-332

Johnston E, Weinstein P, Slaney D, Flies AS, Fricker S, Williams C 2014. Mosquito communities with trap height and urban-rural gradient in Adelaide, South Australia: implications for disease vector surveillance. *Journal of Vector Ecology* 39:48-55

Kay BH, Ferguson KJ, Morgan RNC 1973. Control of salt-marsh mosquitos with abate insecticide at Coombabah-Lakes, Queensland, Australia. *Mosquito News* 33:529-535

Kay BH, Jorgensen WK 1986. Eggs of *Aedes vgilax* (Skuse) and their distribution on plants and soil in south east Queensland saltmarsh. *Australian Journal of Entomology* 25:267-272

Kokkinn MJ, Duval DJ, Williams CR 2009. Modelling the ecology of the coastal mosquitoes *Aedes vigilax* and *Aedes camptorhynchus* at Port Pirie, South Australia. *Medical and Veterinary Entomology* 23:85-91

Kurucz N, Whelan PI, Carter JM, Jacups SP 2009. A geospatial evaluation of *Aedes vigilax* larval control efforts across a coastal wetland, Northern Territory, Australia. *Journal of Vector Ecology* 34:317-323

Miller RJ, Wing J, Cope S, Davey RB, Kline DL 2005. Comparison of carbon dioxide- and octenol-baited encephalitis virus surveillance mosquito traps at the Shoal Water Bay Training area, Queensland, Australia. *Journal of the American Mosquito Control Association* 21:497-500

Ritchie SA 1994. Spatial stability of *Aedes vigilax* (Diptera: Culicidae) eggshells in southeastern Queensland salt marshes. *Journal of Medical Entomology* 31:920-922

Rowbottom R, Carver S, Barmuta LA, Weinstein P, Allen GR 2017. Mosquito distribution in a saltmarsh: determinants of eggs in a variable environment. *Journal of Vector Ecology* 42:161-170

Rowbottom R, Carver S, Barmuta LA, Weinstein P, Foo D, Allen GR 2015. Resource limitation, controphic ostracod density and larval mosquito development. *PLoS one* 10:e0142472

Russell RC, Cloonan MJ, Wells PJ, Vale TG 1991. Mosquito (Diptera: Culicidae) and arbovirus activity on the south coast of New South Wales, Australia, in 1985–1988. *Journal of Medical Entomology* 28:796-804

Russell RC, Vale TG, Wells PJ, Cloonan MJ 1992. Monthly periodicity and abundance of mosquitoes (Diptera: Culicidae) near Batemans Bay on the south coast of New Soth Wales, 1985-1988. *Journal of the Australian Entomological Society* 31:281-288

Russell TL, Gatton ML, Ryan PA, Kay BH 2009a. Quality assurance of aerial applications of larvicides for mosquito control: Effects of granule and catch tray size on field monitoring programs. *Journal of Economic Entomology* 102:507-514

Russell TL, Kay BH, Skilleter GA 2009b. Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. *Aquatic Biology* 6:77-90

Ryan PA, Do KA, Kay BH 2000. Definition of Ross River virus vectors at Maroochy Shire, Australia. *Journal of Medical Entomology* 37:146-152

Ryan PA, Kay BH 1999. Vector competence of mosquitoes (Diptera: Culicidae) from Maroochy Shire, Australia, for Barmah Forest virus. *Journal of Medical Entomology* 36:856-860

Saffigna PG, Dale P 1999. Acid sulfate soils in intertidal mosquito breeding habitats and implications for habitat modification. *AGRIS* 15:520-525

Shinkarenko L, Hulsman K, Mottram P, Dale P, Kay BH 1986. Reliability of using head capsule width and body length to identify larval instars of *Aedes vigilax* (Diptera: Culilcidae). *Australian Journal of Entomology* 25:37-40

Turner PA 2002. Relationship between age and colour of hatched eggshells of *Ochlerotatus vigilax* (Skuse) (Diptera: Culicidae) on an Australian saltmarsh. *Australian Journal of Entomology* 41:324-328

Turner PA, Streever WJ 1997. The relationship between the density of *Aedes vigilax* (Diptera: Culicidae) eggshells and environmental factors on Kooragang Island, New South Wales, Australia. *Journal of the American Mosquito Control Association* 13:361-367

Turner PA, Streever WJ 1999. Changes in productivity of the saltmarsh mosquito, *Aedes vigilax* (Diptera: Culicidae), and vegetation cover following culvert removal. *Australian Journal of Ecology* 24:240-248

van Schie C, Spafford H, Carver S, Weinstein P 2009. Salinity tolerance of *Aedes camptorhynchus* (Diptera: Culicidae) from two regions in southwestern Australia. *Australian Journal of Entomology* 48:293-299

Walker LJ, Selvey LA, Jardine A, Johansen CA, Lindsay MDA 2018. Mosquito and virus surveillance as a predictor of human Ross River virus infection in south-west Western Australia: how useful is it? *American Journal of Tropical Medicine and Hygiene* 99:1066-1073

Webb CE, Russell RC 1999. Towards management of mosquitoes at Homebush Bay, Sydney, Australia. I. Seasonal activity and relative abundance of adults of *Aedes vigilax, Culex sitiens,* and other salt-marsh species, 1993-94 through 1997-98. *Journal of the American Mosquito Control Association* 15:242-249

Webb G, Miller P, Peters B 2012. Pyripoxyfen for the control of Australian salt-marsh mosquito, *Aedes vigilax*. *Journal of the American Mosquito Control Association* 28:50-52

Williams CR, Bader CA, Williams SR, Whelan PI 2012. Adult mosquito trap sensitivity for detecting exotic mosquito incursions and eradication: a study using EVS traps and the Australian southern saltmarsh mosquito, *Aedes camptorhynchus*. *Journal of Vector Ecology* 37:110-116

Williams CR, Williams SR, Nicholson J, Little SM, Riordan J, Fricker SR, Kokkinn MJ 2009a. Diversity and seasonal succession of coastal mosquitoes (Diptera: Culicidae) in the northern Adelaide region of South Australia. *Australian Journal of Entomology* 48:107-112

Williams SR, Kokkinn MJ, Fricker SR, Schneemilch MJ, Humphrey MJ, Williams CR 2009b. Mosquitoes (Diptera: Culicidae) of the Spencer Gulf Coast of South Australia. *Transactions of the Royal Society of South Australia* 133:51-56

Yang GJ, Brook BW, Whelan PI, Cleland S, Bradshaw CJA 2008. Endogenous and exogenous factors controlling temporal abundance patterns of tropical mosquitoes. *Ecological Applications* 18:2028-2040

Chapter 3

Chapter 3 – How do local differences in saltmarsh ecology influence disease vector mosquito populations?

Raylea Rowbottom [a], Scott Carver [b], Leon A Barmuta [b], Philip Weinstein [c], Geoff R Allen [a]

[a] Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Australia

[b] School of Natural Sciences (Biological Sciences), University of Tasmania, Hobart, Australia

[c] School of Biological Science, University of Adelaide, Adelaide, Australia

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Author contributions: RR, SC, GA. conceived the design of the project. RR, SC, GA and PW conducted the field work. RR processed all the laboratory work. LB and RR conducted the statistical analysis. GA oversaw the project and provided conceptual guidance. RR wrote the manuscript with critical feedback from all authors.

3.0 Abstract

Saltmarsh breeding mosquitoes are an important source of vectors for arboviral transmission. In southern Australia the most prominent vector-borne disease, Ross River virus (Togaviridae: *Alphavirus*) (RRv) is transmitted by the saltmarsh mosquito (Diptera: Culicidae) *Aedes camptorhynchus* (Thomson). However, factors driving abundance of this mosquito within and among saltmarshes are poorly understood. To predict the abundance of this mosquito within saltmarshes we monitored the environmental conditions and aquatic invertebrate ecology of three temperate saltmarshes habitats over two seasons. Up to 44% of first instar mosquito numbers and 21% of pupal numbers were accounted for by environmental variables. Samphire vegetation cover was a common predictor of first instar numbers across sites although, between saltmarshes, aquatic factors such as high salinity, temperatures less than 22°C and water body volume were important predictors. Identified predictors of pupal numbers were more variable and included high tides, waterbody volume and alkalinity. The composition of invertebrate functional feeding groups differed between saltmarshes and showed that increased diversity led to fewer mosquitoes. It was evident that seemingly similar saltmarshes can vary markedly in invertebrate assemblages, water availability and conditions through tidal inundations, rainfall or waterbody permanency. This study advances insight into predictors of vector mosquito numbers that drive the risk of RRv outbreaks.

Keywords

Aedes camptorhynchus, functional feeding groups, habitat comparison, RRv, seasonal variability, Tasmania

3.1 Introduction

Saltmarshes are an important source of halotolerant mosquitoes in Australia and many other parts of the world (Russell and Dwyer 2000, Gubler 2002). The two principal saltmarsh mosquito vectors along coastal regions across Australia are *Aedes vigilax* (Skuse) and *Ae. camptorhynchus* (Thomson), which are responsible for the majority of Ross River virus (RRv) outbreaks - Australia's most important vector-borne disease (Russell 1998). Research has been directed towards understanding the life history and development of these mosquitoes emerging from saltmarsh habitats (Bi et al. 2003, de Little et al. 2009, Yang et al. 2009, Werner et al. 2012). However, less is understood about predictors of mosquito abundance in relation to both aquatic environmental parameters and other invertebrate communities occupying saltmarshes and how comparably different these seemingly simple ecosystems are in space and time (Carver et al. 2010a). Enhancing our knowledge of saltmarsh ecology may have important consequences for understanding the dynamics of vector populations and the changes in biotic and abiotic factors that regulate vector mosquito abundance, and therefore the risk of outbreaks of RRv.

Saltmarshes are generally ecologically simple environments, owing to low species richness of plants and animals (Adam 1990, Saintilan 2009). Nevertheless, differences in both environmental conditions and invertebrate assemblages occur among saltmarshes that are seemingly similar, and these can influence mosquito populations. For example, variation in the nature and number of aquatic competitors and predators in saltmarshes has been reported to influence mosquito oviposition (Mokany and Shine 2003, Eitam and Blaustein 2004, Vonesh and Blaustein 2010) thereby altering the spatial distribution of mosquito assemblages. Research also has shown that presence of competitors and predators within aquatic environments can impede mosquito larval development resulting in reduced larval survival, slower developmental rate and smaller adult mosquitoes (Knight et al. 2004, Alto et al. 2005, Vonesh

and Blaustein 2010, Farjana et al. 2012, Rowbottom et al. 2015, Rowbottom et al. 2017). Similar changes to mosquito development, size and survival can also be observed with environmental stress, such as increases in water temperatures and salinity and eutrophication (Clark et al. 2004, van Schie 2006, Kokkinn et al. 2009, van Schie et al. 2009, Williams et al. 2009, Chaves et al. 2011, Muturi et al. 2011), which can, in turn, influence vector competence, and virus transmission intensity (Paaijmans et al. 2012).

Given that saltmarshes occur in an array of different regions, from coastal, to inland lagoons and estuaries, it is important for local public health management to understand variation in conditions and driving forces behind mosquito assemblages within them. In some cases, variation of taxa has been observed over small spatial scales with habitats of a range of sizes, differences in surrounding land use, and with changes in vegetation types (Pinder et al. 2005, Banerjee et al. 2010, Carver et al. 2010b, Roiz et al. 2015, Haynert et al. 2017). An appreciation for the invertebrate community structure and the local environmental processes that vary among saltmarshes will enlighten understanding of spatially varying mosquito abundance and potentially vector-borne disease (Wisz et al. 2013).

Epidemiologically, RRv causes the most commonly reported mosquito-borne disease, epidemic polyarthritis, in Australia, averaging 4800 clinical notifications a year (NNDSS 2019). Outbreaks of RRv are complex and difficult to predict, involving local knowledge of vectors, aquatic conditions and the zoonotic reservoir host populations (Claflin and Webb 2015). In Australia, the majority of RRv notifications occur in proximity to saltmarsh habitats, where the virus is largely vectored by *Ae. camptorhynchus* in the south and *Ae. vigilax* north of the range of *Ae. camptorhynchus*. In southern Tasmania, the location of this study, *Ae. camptorhynchus* is responsible for infecting an average of 26 people per annum, with outbreaks years reaching 117, and cases occurring predominantly in suburbs adjoining saltmarshes (Robertson et al. 2004, Carver et al. 2011, Rowbottom et al. 2017). *Aedes camptorhynchus*

rapidly colonises waterbodies after significant rainfall and tidal inundations and is capable of tolerating salinities exceeding that of sea water (Carver et al. 2009, van Schie et al. 2009). Other aquatic conditions, such as pH and the presence of predators, have been recorded to influence *Ae. camptorhynchus* numbers (Carver et al. 2009, Carver et al. 2010b). At present, little is understood about the ecology of *Ae. camptorhynchus* within temperate saltmarsh habitats, the taxonomic diversity within these habitats that may influence this mosquito's abundance, and how this varies amongst saltmarshes.

The aims of this study are to compare the ecology of temperate saltmarshes by studying three environmentally distinct saltmarsh habitats. Our objective is to: (1) to explore differences in invertebrate functional feeding groups within the saltmarsh aquatic environments; and (2) to quantify aquatic invertebrate diversity and environmental conditions in relation to mosquito vector abundance over the immature lifecycle stages of mosquitoes. By doing so our goal is to identify key ecological processes that may impact vector abundance in temperate saltmarshes, and thereby potentially inform the targeted management of RRv.

3.2 Materials and Methods

3.2.1 Field Sampling

This study was conducted at three saltmarshes that were selected for having differing tidal and environmental parameters in Southern Tasmania; Primrose Sands (42.88° S, 147.66° E), Cambridge (42.38° S, 147.49° E,) and Richmond (42.76 S, 147.45° E) (Figure 3.1). These regions of Tasmania are close to the Hobart population centre and are known for seasonally large populations of *Ae. camptorhynchus* (Robertson et al. 2004, Carver et al. 2011). The Primrose Sands saltmarsh is situated on the Carlton River, a saline and tidal area of the river near its terminus into the ocean. Both the Cambridge and Richmond saltmarshes are situated

on the Coal River. The Cambridge saltmarsh is tidally influenced and is close to the river terminus into Frederick Henry Bay. The Richmond saltmarsh is 12 km upstream from Cambridge towards the river headlands, although still saline and tidally influenced.

Figure 3.1: Map of Tasmania showing the population centre (red dot) with the locations of the three saltmarshes expanded, A, Primrose, B, Cambridge, C, Richmond.

Light Detection and Ranging (LiDAR) data were acquired on the 2nd October 2012 during a phase of neap tides, whereby no tidal inundation occurred at the saltmarshes. A digital elevation model of the three saltmarshes was derived from LiDAR ground-only point data with a pixel spacing of 1 m using the package 'raster' (version 2.9-23) in R (version 3.3.3) (R Core Team 2019). Tidal lines were overlaid on the maps with ArcGIS (ESRI 2014) (Figure 3.2).

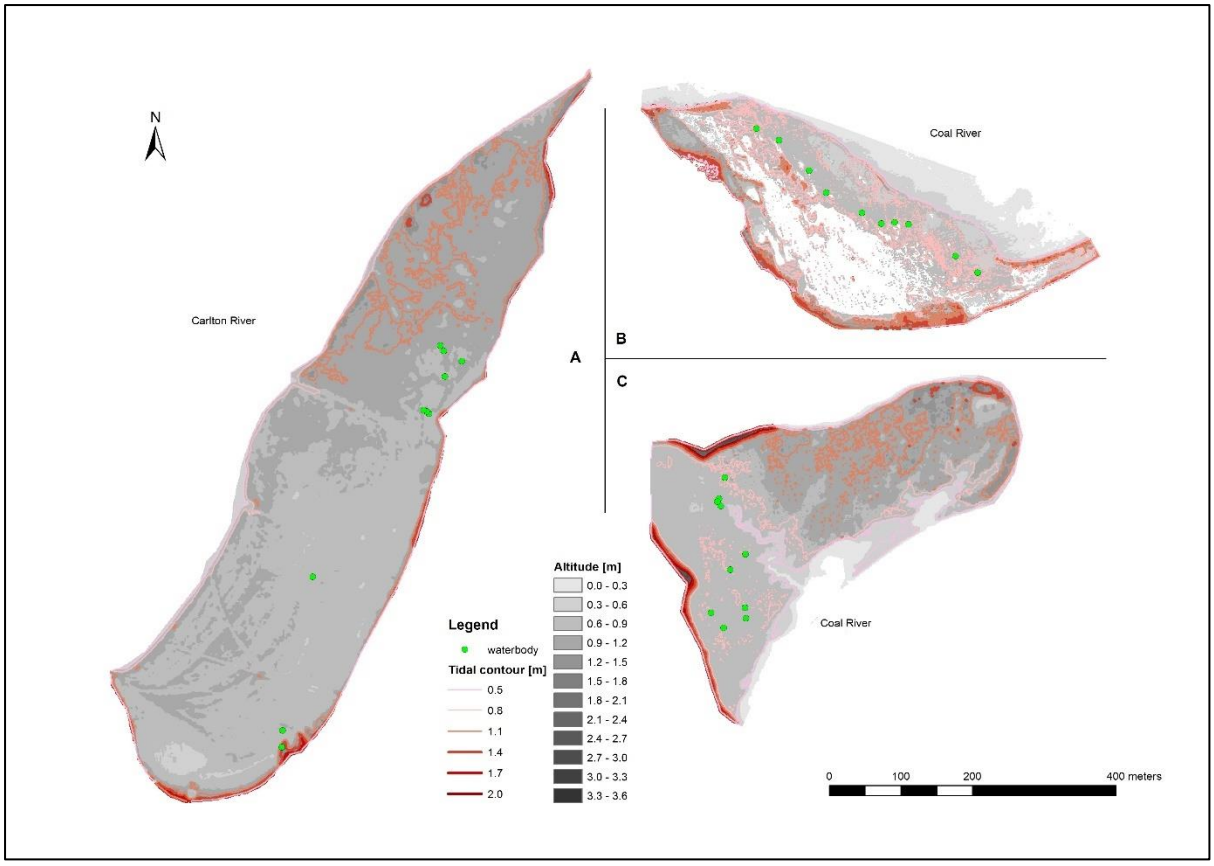


Figure 3.2: Digital elevation models of the three saltmarshes showing grey-shaded elevation (meters) with coloured contour lines indicating inundation markers of tidal heights (m). Green dots indicate the ten waterbodies sampled within each site.

The three saltmarshes were sampled over the peak season of RRv and mosquito abundance in Tasmania (October-March, 2011-2012 and 2012-2013) (Werner et al. 2012). From each saltmarsh, ten waterbodies, distributed across the entire saltmarsh, were opportunistically selected for repetitive weekly sampling. Each week the volume of the waterbodies was calculated by measuring the maximum length, width and five depth measurements taken at random. In addition, aquatic environmental parameters were measured for the given depth of each water body at the time of measurements which included; pH, temperature (°C), and salinity (ppt) using a handheld instrument (YSI Incorporated; Model 63 Handheld pH, Conductivity, Salinity and Temperature System, Yellow Springs, Ohio. www.YSI.com). For

invertebrate sampling we used a 125 mm diameter mosquito larval dipper (Australian Entomological Supplies, E920). Individual dips were then poured through a 99 µm (0.0039 inch) sieve and backwashed into the 50 mL screw cap vials, numbered, and returned to the laboratory for processing.

Daily precipitation data (mm) were obtained from the Australian Bureau of Meteorology (BOM). For all sites the closest BOM station was chosen for precipitation data. Spatially matched sites included: Sorell (Abbatoirs) (42.74° S, 147.45° E) for Primrose Sands, Hobart Airport for Cambridge (42.83° S, 147.50° E), and Richmond (Lowlands) for Richmond (42.74° S, 147.45° E).

Daily maximum and minimum temperatures (°C) were also obtained by the Australia Bureau of Meteorology. As the Bureau of Meteorology has no temperature recording sites near all three locations, we used a single temperature monitoring station that is closest to all collection sites. The site is Campania (Kincora) (42.69° S, 147.43° E) approximately 27.65 km South East from Primrose Sands, 15.80 km south from Cambridge and 7.15 km from Richmond saltmarshes.

Daily tidal data were collected from the Bureau of Meteorology containing tidal height in metres. The tidal station used for all sites was Hobart (42.88° S, 147.33° E).

3.2.2 Laboratory processing of samples

At the completion of weekly collections, samples were poured through a 99 µm (0.0039 inch) sieve and backwashed into the screw cap vials using ethanol (70%) and stored in a refrigerator (Austral Insulation A/N 2181) at 4°C until processed. Samples were processed in quartered Petri dishes under a dissecting microscope (Nikon: SMZ800). For samples with large numbers of invertebrates, a single quarter was assessed for all invertebrates and multiplied by four (Mogi

and Okazawa 1990). All culicids were identified to species, other dipterans to family and all remaining invertebrates identified to order.

3.2.3 Statistical analysis

3.2.3.1 Differences in environmental conditions & functional feeding groups (FFG)

We investigated how environmental conditions varied between the saltmarshes and if these differences relate to saltmarsh community composition. Firstly, we assigned all invertebrates scored from field samples into functional feeding groups (FFGs): filter feeders, predators, collector & gatherers, shredders and grazers with mosquitoes assigned to a separate group (Cummins and Klung 1979, Ford et al. 2013, Ramírez and Gutiérrez-Fonseca 2014, Alexandridis et al. 2017) (Table 3.1). Changes in community composition in each water body were visualised using unconstrained ordinations (non-metric multidimensional scaling – NMDS – based on Bray-Curtis dissimilarity measures) computed from square-root transformed abundance data using the function 'metaMDS' in the 'vegan' package (Oksanen 2019). The relationships with environmental predictor variables and the dissimilarities were tested using permutational ANOVA (marginal tests from the 'adonis2' function in 'vegan' using waterbodies as blocks to account for repeated sampling; 5000 permutations). Significant relationships were plotted as vectors on the unconstrained ordinations. Secondly, we explored how the overall invertebrate composition of waterbodies changed between sites and sampling seasons by computing median abundance for each FFG and larval mosquito for each waterbody across sampling dates within each sampling season.

Table 3.1: Invertebrates identified from waterbody sampling divided into their functional feeding groups (FFG).

FFG	Order	Family
Collector & Gatherer	Amphipoda	
	Diptera	Stratiomyidae
	Diptera	Ceratopogonidae
	Diptera	Ephydrididae
	Diptera	Chironomidae
	Diptera	Psychodidae
Filter feeder	Podocopida (Class: Ostracoda)	
	Cyclopoida (Subclass: Copepoda)	
	Cladocera	
	Anostraca	
	Diptera	Simuliidae
Predator	Diptera	Sciomyzidae
	Diptera	Athericidae
	Diptera	Empididae
	Diptera	Dolichopodidae
	Diptera	Tipulidae
	Hemiptera	Gerridae
	Acarina	
Shredders & Grazers	Isopoda	
	(Class Gastropoda)	
	(Class Collembola)	

3.2.3.2 Quantification of environmental conditions in relation to vector mosquitoes

Firstly, we excluded any sample dates for a given waterbody if the waterbody was dry. We focussed the analysis on first instar and pupal mosquitoes as these are the most important determinants of hatching success and recruitment to adults, respectively. Temporal lags account for effects of environmental predictors on the lifecycle of vector mosquitoes. Therefore, we determined the optimal time-lags between environmental predictors and mosquito abundance using cross-correlation analysis for each environmental variable at each site (Table 3.2). Cut-off points for maximum lags used were 5 d for first instar larvae and 30 d for pupae (Barton and Aberton 2005, Dale and Breitfuss 2009). Additionally, we computed the accumulated rainfall (mm), maximum temperature (°C) and maximum tidal height (m) prior to peak mosquito observations for each site.

Table 3.2: Number of lag days for each environmental predictor at each site for first instar (I) and pupae (P).

Variable	Primrose Sands	Cambridge	Richmond
Rainfall (mm)			
I	-5	-1	-1
P	-12	-13	-12
Temperature (°C)			
I	-5	-3	-3
P	-20	-2	-20
Tide (m)			
I	-5	-2	-2
P	-13	-4	-4

All environmental variables were checked by scatterplot matrices for multi-collinearity; where there were high correlations ($|r| > 0.7$), the more biologically relevant variable was retained, and the redundant variable removed prior to analysis. The maximum correlation between retained environmental variables was $r = 0.42$.

Because the randomisation tests used above only detect, at best, linear or monotonic relationships, we used machine learning techniques to identify the best predictive models of mosquito abundance. We used Random Forests (RF), which constructs multiple regression trees (non-linear models) using bootstrapped training samples from the original data and outputs an average of their prediction results (Breiman 2001). Within a tree, each node considers a randomized subset of the original variables. Bootstrapping, variable randomization and averaging are critical features that make RF robust, especially for analysis of complex, nonlinear and highly dimensional data (Cutler et al. 2007). We used Increased Mean Square Error (IncMSE) as a measure of the prediction accuracy of the RF model. If the random permutation drastically changes the predicted value (as measured by the IncMSE), then the original variable is considered critical. We set the number of trees at 5000 for each model and tuned the random forests to minimise out of bag (OOB) error for first instars and pupae for each site separately. Approximately one-third ($1/e = 0.3678$: Breiman 2001) of samples were OOB in each training set. All random forests were fitted using the 'randomForest' package (Liaw and Wiener 2002) in R version 3.5.3 (R Core Team 2019). The best-fit forests were then interpreted using the Interpretable Machine Learning ('iml') package (Molnar et al. 2018). To visualise the relationships between the response variables and the two most important predictor variables, we used 30 randomisations of the predictor variables of each RF model using accumulated local effects (ALE) plots. For all important predictor variables individual conditional expectation (ICE) plots were inspected.

3.3 Results

3.3.1 Differences in environmental conditions & functional feeding groups (FFG)

We collected a total of 6,300 samples from 30 waterbodies, ten waterbodies at each of the three saltmarshes during the collection periods. The significant environmental predictors for FFG assemblage across the waterbodies for the three saltmarshes were: salinity ($F_{(2, 763)} = 9.434$, $p < 0.002$), rainfall ($F_{(2, 763)} = 10.093$, $p < 0.001$), pH ($F_{(2, 763)} = 5.390$, $p < 0.001$), temperature ($F_{(2, 763)} = 36.023$, $p < 0.001$), number of days waterbody was wet ($F_{(2, 763)} = 17.867$, $p < 0.001$) and samphire vegetation cover ($F_{(2, 763)} = 18.736$, $p < 0.001$). Over both seasons, Cambridge had the greatest number of filter feeders and these assemblages were influenced by pH and longer periods of waterbodies remaining wet (Table 3.3). Richmond saltmarsh waterbodies had the greatest overall abundance of FFG and waterbodies were on average warmer than both Primrose Sands and Cambridge with lower levels of salinity (Table 3.3 and Figure 3.3). Greater numbers of mosquitoes were observed for both seasons at Primrose Sands compared to the other two saltmarshes, with abundance being driven by higher average rainfall and greater samphire cover (Figure 3.3). Seasonal composition of invertebrates within waterbodies differed ($F_{(2, 763)} = 9.254$, $p < 0.001$) with environmental predictors fluctuating across the two collection seasons (Table 3.3).

Table 3.3: Environmental variables for the two seasons (2011-2012 and 2012-2013) that were significant predictors of functional feeding group (FFG) assemblage at the three saltmarshes; Primrose Sands, Cambridge and Richmond, Tasmania. Seasonal ranges of salinity, rainfall, pH, temperature and number of days waterbodies were wet data include averages ± SD. Numbers in parenthesis equal the sample size.

Variable	2011-2012			2012-2013		
	Primrose Sands	Cambridge	Richmond	Primrose Sands	Cambridge	Richmond
Salinity (ppt)	12.3–89.4	10.0–75.8	19.9–79.2	11.5–101.3	11.6–90.4	13.1–72.5
Mean ± SD	49.0 ± 14.0 (115)	12.9 ± 50.9 (170)	12.5 ± 47.2 (127)	18.0 ± 52.7 (109)	15.6 ± 57.9 (159)	12.9 ± 49.8 (130)
Rainfall (mm)	0–15	0–15	0–15	0–10	0–10	0–3
Mean ± SD	1.6 ± 4.0 (40)	1.4 ± 3.8 (52)	1.6 ± 4.0 (42)	1.5 ± 2.7 (55)	1.3 ± 2.6 (62)	0.6 ± 0.9 (64)
pH	6.2–10.0	6.4–9.6	6.2–10.4	5.9–9.3	6.2–9.2	5.4–9.4
Mean ± SD	7.7 ± 0.2 (115)	7.5 ± 0.6 (170)	7.8 ± 0.3 (127)	7.6 ± 0.1 (109)	7.1 ± 0.6 (159)	7.2 ± 0.3 (130)
Temperature (°C)	11.4–29.4	12.0–31.7	13.7–33.2	11.1–33.3	12.4–34.9	13.1–40
Mean ± SD	19.0 ± 4.6 (115)	21.9 ± 4.2 (170)	23.6 ± 5.1 (127)	20.0 ± 4.8 (109)	22.2 ± 4.5 (159)	23.1 ± 5.5 (130)
# days wet	7–91	7–49	7–42	7–56	7–56	7–42
Mean ± SD	19.5 ± 16.1 (115)	22.5 ± 12.3 (170)	14.7 ± 7.9 (127)	17.9 ± 11.3 (109)	21.97 ± 13.9 (159)	17.1 ± 9.6 (130)

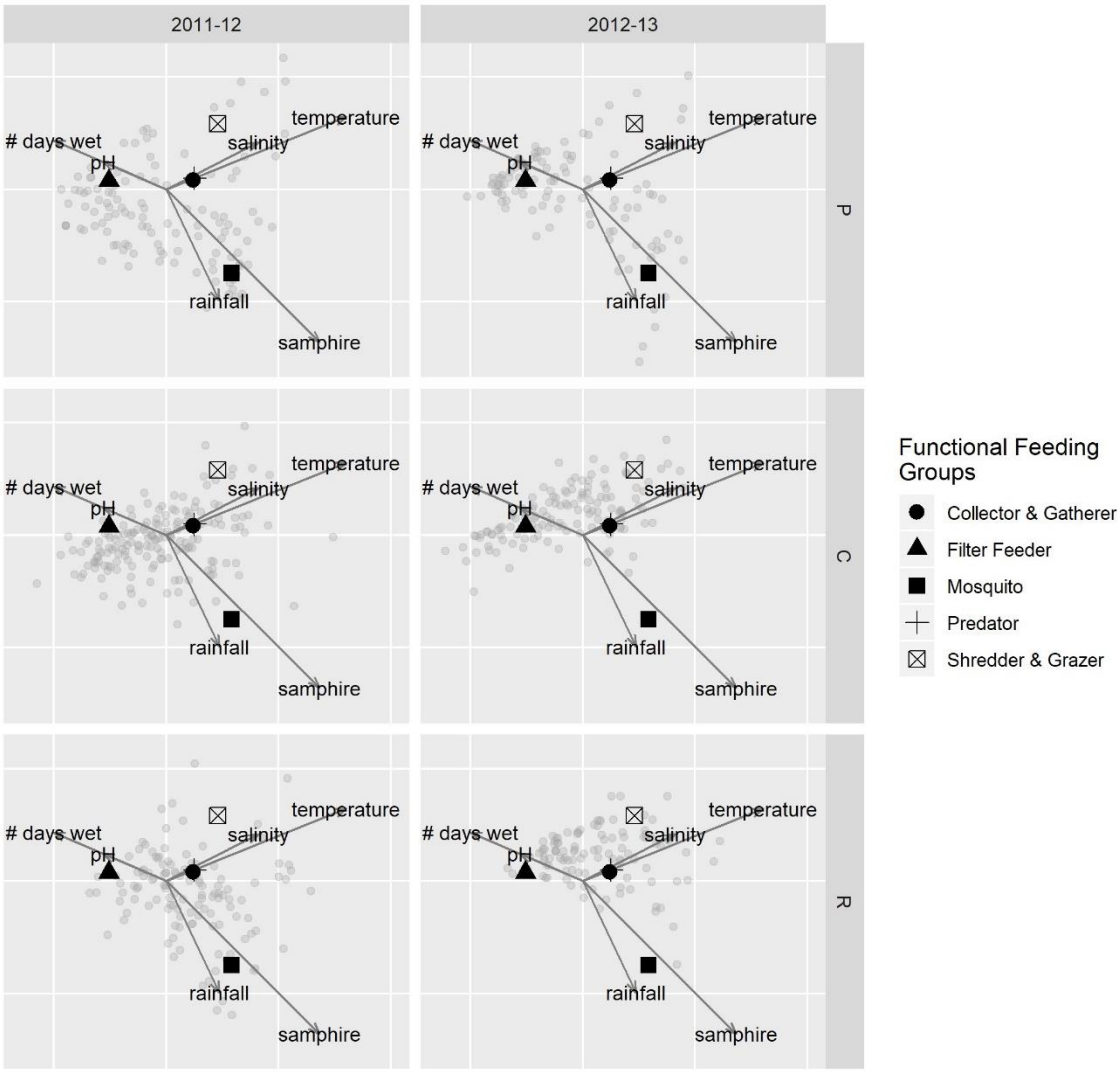


Figure 3.3: Non-metric multidimensional scaling (NMDS) ordination plots displaying both collection seasons (2011-12 and 2012-13) and site (Primrose Sands (P), Cambridge (C), and Richmond (R)). Plots include axis of significant predictor variables ($p>0.05$), with response variables as symbols overlaid on the plot. NMDS stress = 0.128.

Both season and site differed in relation to FFG assemblage ($F_{(2, 763)} = 16.501$, $p < 0.001$ and $F_{(2, 763)} = 8.780$, $p < 0.001$, respectively) (Table 3.3). Overall, the first season had relatively more mosquitoes than the second season, with the second season being dominated by filter feeders and collector & gatherers. Composition of invertebrates remained relatively constant over both seasons for the Cambridge saltmarsh, whereas fewer predators and collector & gatherers were observed in the second season for the Richmond saltmarsh. Primrose Sands invertebrates shifted from mosquitoes in the first season towards filter feeders in the second season (Figure 3.4).

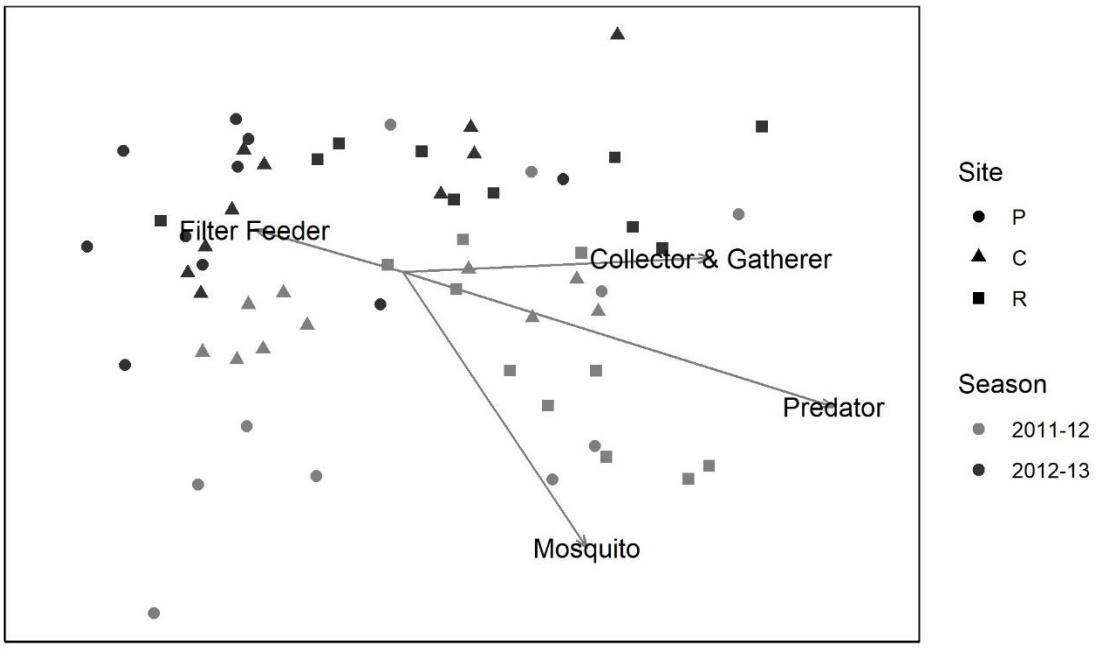


Figure 3.4: Non-metric multidimensional scaling (NMDS) ordination plot displaying the changes in functional feeding groups for both seasons (2011-12 (light grey) and 2012-13 (dark grey)) and between the three collection sites (Primrose (circle), Cambridge (triangle) and Richmond (square)). Plots include axis of significance, with response variables as symbols overlaid on the plot. NMDS stress = 0.098

3.3.2 Quantifying environmental conditions in relation to vector mosquitoes

Moderate amounts of variability were accounted for among sites and instars. For both Primrose Sands and Richmond saltmarshes, greater variability in mosquito numbers was explained in first instars (28.5% and 44.33%, respectively), relative to pupae (11.68% and 20.62%), whereas more variability was explained for pupae at Cambridge (17.46%) than first instars (12.65%). Samphire (*Sarcocornia quinqueflora* [Bunge ex Ung.-Sternb.] A. J. Scott) cover was the common predictor variable for abundance of first instars, with pupae having little similarity in predictor variables across sites. Although, no sites shared the same set of predictor variables for both first instars and pupae (Figure 3.5 and Figure 3.6).

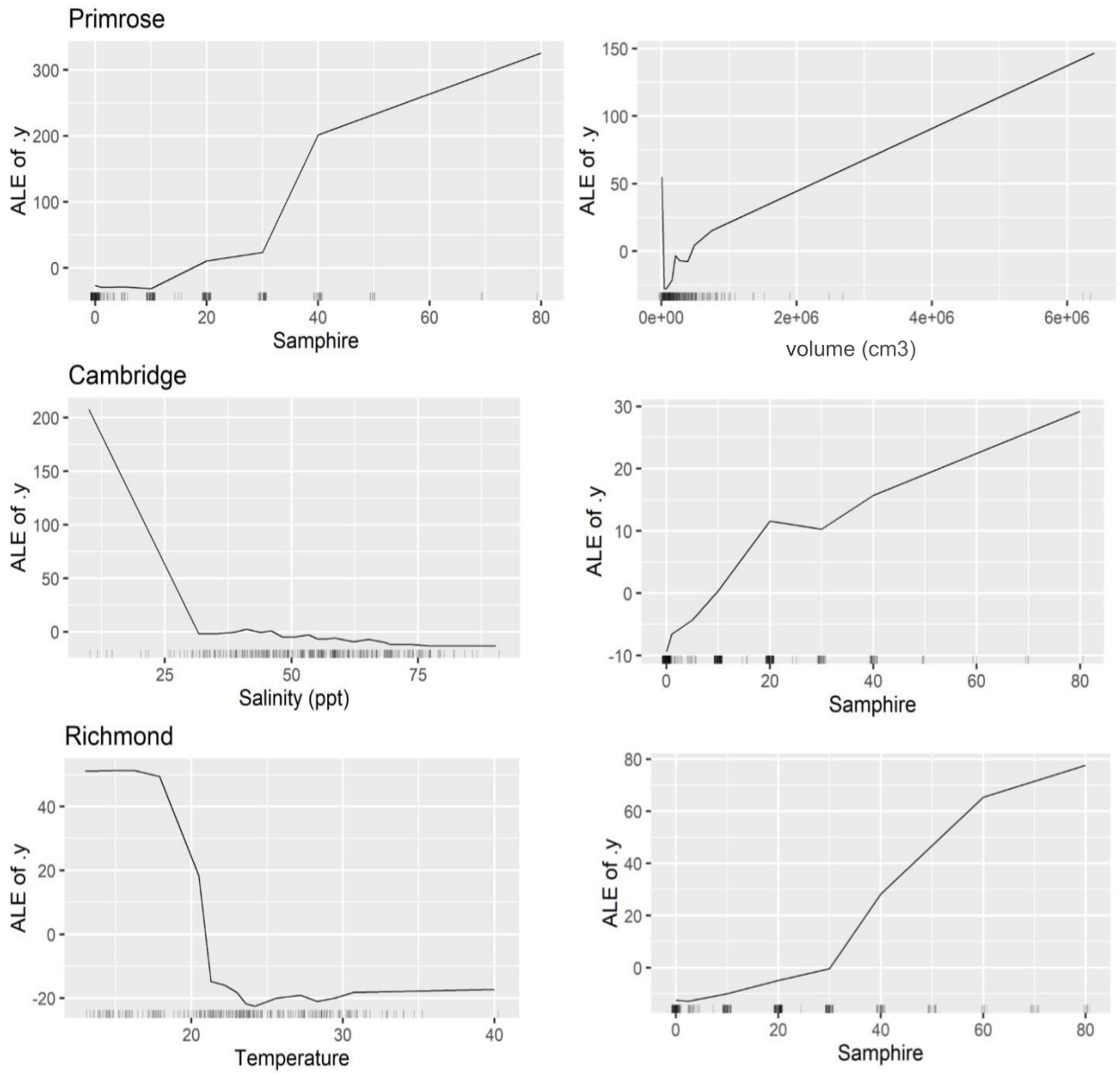


Figure 3.5: Accumulated local effects (ALE) partial dependence plots for the two most important predictor variables, at each site, for the abundance of first instars. Mosquito first instar abundance is indicated on the y-axis.

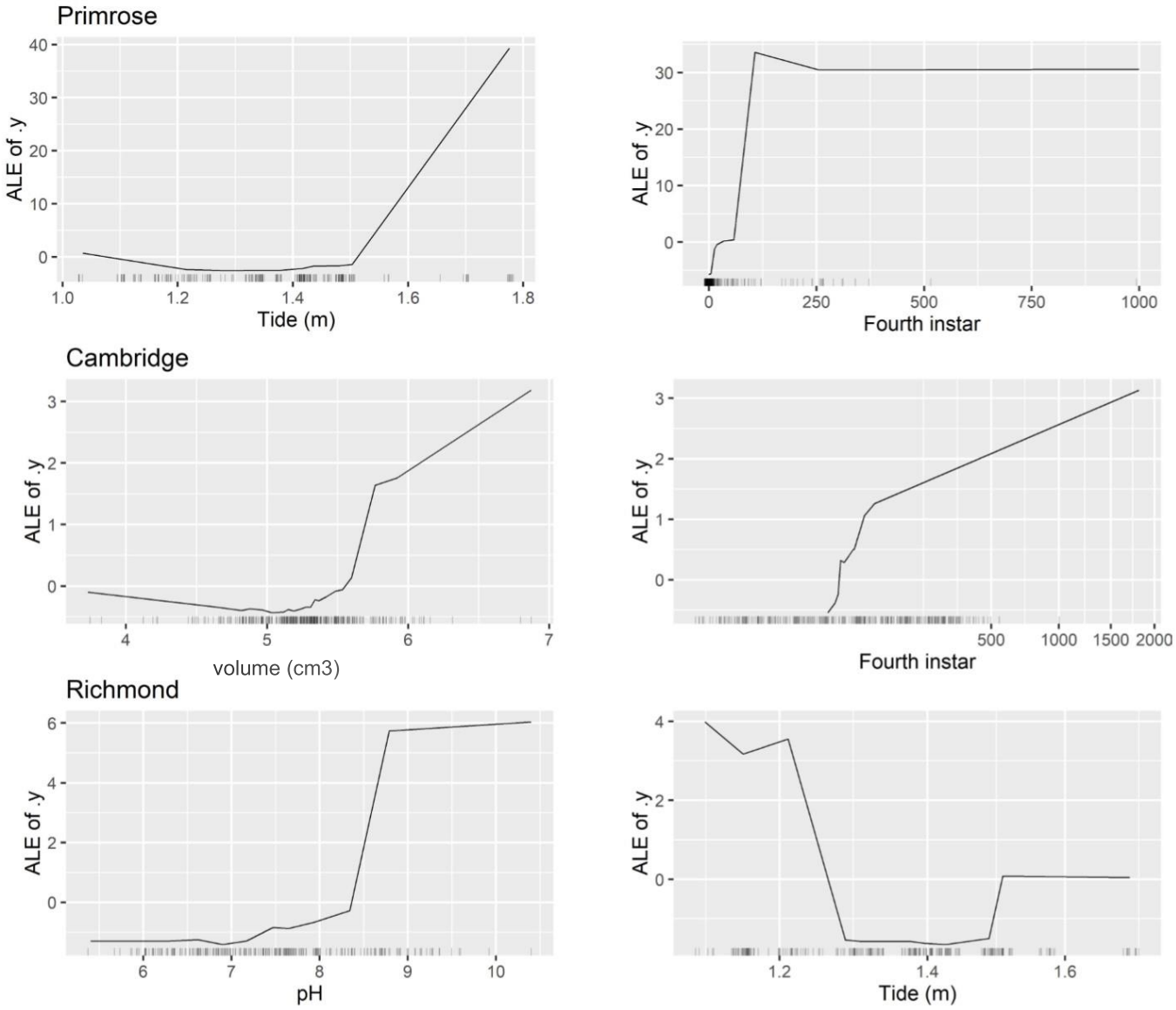


Figure 3.6: Accumulated local effects (ALE) partial dependence plots for the two most important predictor variables, at each site, for the abundance of pupae. Pupae abundance is indicated on the y-axis

Greater numbers of first instar larvae were observed in the Primrose Sands saltmarsh with between 10% and 40% cover of samphire, followed by waterbodies with volumes of approx. 7 litres. Similarly, samphire cover between 15% and 40% was the second primary predictor for number of first instar larvae at both Cambridge and Richmond saltmarshes. The top ranked environmental predictor for Cambridge was high salinity conditions (40-50 ppt), whereas for Richmond saltmarsh, greater numbers of first instar larvae were observed with temperatures between 18 and 22°C (Figure 3.5), with a sharp decline in numbers at temperatures greater than 22°C and very few past 30°C.

Tide was an important predictor for Primrose Sands with higher tides (>1.3 m) predicting numbers of pupae. The other important predictor at Primrose Sands was the presence of fourth instar larvae, with higher numbers of fourth instar larvae predicting the abundance of pupae. Cambridge saltmarsh also had a greater number of pupae present when higher numbers of fourth instar were recorded, with increased waterbody volume (>5 cm^3) as the primary prominent predictor for pupae. Alkaline conditions (pH 7.5-8.3) predicted more pupae in the Richmond saltmarsh and greater numbers of pupae were present when tides were between 1.2 and 1.3 m (Figure 3.6).

3.4 Discussion

Some of the most important arboviruses can be transmitted by saltmarsh derived mosquitoes such as; RRv and Barmah Forest virus (Russell 1998). Within Australia, the primary saltmarsh vectors responsible for virus transmission of epidemiological importance include *Ae. vigilax* and *Ae. camptorhynchus.* In this study, we focused on comparing the differences in ecology between three superficially similar local saltmarsh habitats in southern Tasmania, known to harbour vector mosquitoes. We found each of these superficially similar saltmarshes to be

unique in both environmental conditions and aquatic diversity. We revealed that environmental predictors of presence of immature mosquitoes differed between first instar and pupae and that sites and abundance was impacted by changes in FFG assemblage. Also, assemblages of FFG were affected by environmental conditions with the presence of distinct groups changing between the saltmarshes.

Among our three study sites we observed significant differences in FFG assemblages relating to changes in environmental conditions. Out of the three saltmarshes, the Primrose Sands saltmarsh has experienced the greatest anthropogenic disturbance, from vehicle traffic, rubbish dumping and man-made grid ditching, a technique used to drain saltmarsh habitats for agricultural use (*pers comm* G. Roberts). Of the three sites, Primrose Sands had the highest mosquito abundance and lowest taxonomic diversity. It has long been recognised that human interference in fragile environments has lasting impacts particularly for ecosystem health (Weinstein 1996). Analogous to the Primrose Sands site, populations of *Ae. vigilax* have also increased in degraded mangrove habitats (Claflin and Webb 2017). In comparison, both Cambridge and Richmond saltmarshes, are classified as Ramsar sites because of their high conservation value, with the Richmond saltmarsh also being a wildlife protected region under the Protected Areas on Private Land Program (Prahalad and Pearson 2013). Both these sites had greater representation of other FFGs, which reflects the overall health of these saltmarsh ecosystems (Cadotte et al. 2011). Therefore, indicating that increased biodiversity and habitat health are some of the key factors underlying the RRv vector in these systems (O'Sullivan et al. 2008).

The topography of saltmarshes can impact environmental conditions, particularly aquatic habitats via the frequency of tidal inundations. A study by Richardson et al. (1998) investigating crustacean diversity in saltmarshes described saltmarsh topography, particularly elevation, as the strongest influence on crustacean assemblage due to water permanency. This

physical feature of the Primrose Sands saltmarsh is a contributing factor to the differences observed in waterbody environmental conditions compared to Cambridge and Richmond saltmarshes. Most of the perimeter of the saltmarsh sits between 1.5-2.0 meters high, therefore, inundations of the marsh seldom occur with regular daily high tides being insufficient to breach the banks of the saltmarsh. This and the relatively low levels of rainfall during our study resulted in waterbodies evaporating, increasing the salinity to levels that are incompatible to many aquatic invertebrates (Evin and Talley 2000, Silberbush et al. 2005, Carver et al. 2009). As such, these conditions resulted in an absence of many other FFG and consistently high numbers of mosquitoes, particularly *Ae. camptorhynchus* as they can tolerate quite saline conditions (van Schie et al. 2009, Bader and Williams 2011). By comparison, both the Cambridge and Richmond saltmarshes, despite their relative similarity in elevation, had dissimilar aquatic environmental conditions. Like Talley and Talley (2008), who report declining salinity with distance from the sea, we observed lower salinity levels at the Richmond saltmarsh, as well as warmer waterbodies. These conditions were favourable to predators and collector and gatherers, which have been shown to prefer less saline habitats and are also likely driving the relatively lower presence of mosquitoes observed at this site (Carver et al. 2010b). In comparison, the Cambridge saltmarsh waterbodies had more consistent neutral pH with greater retention of water than both Richmond and Primrose Sands. Water persistency is an important element for many aquatic invertebrates, especially filter feeder survival, particularly ostracods and cladocerans (Eitam et al. 2004), which were the dominant class of filter feeders found at Cambridge.

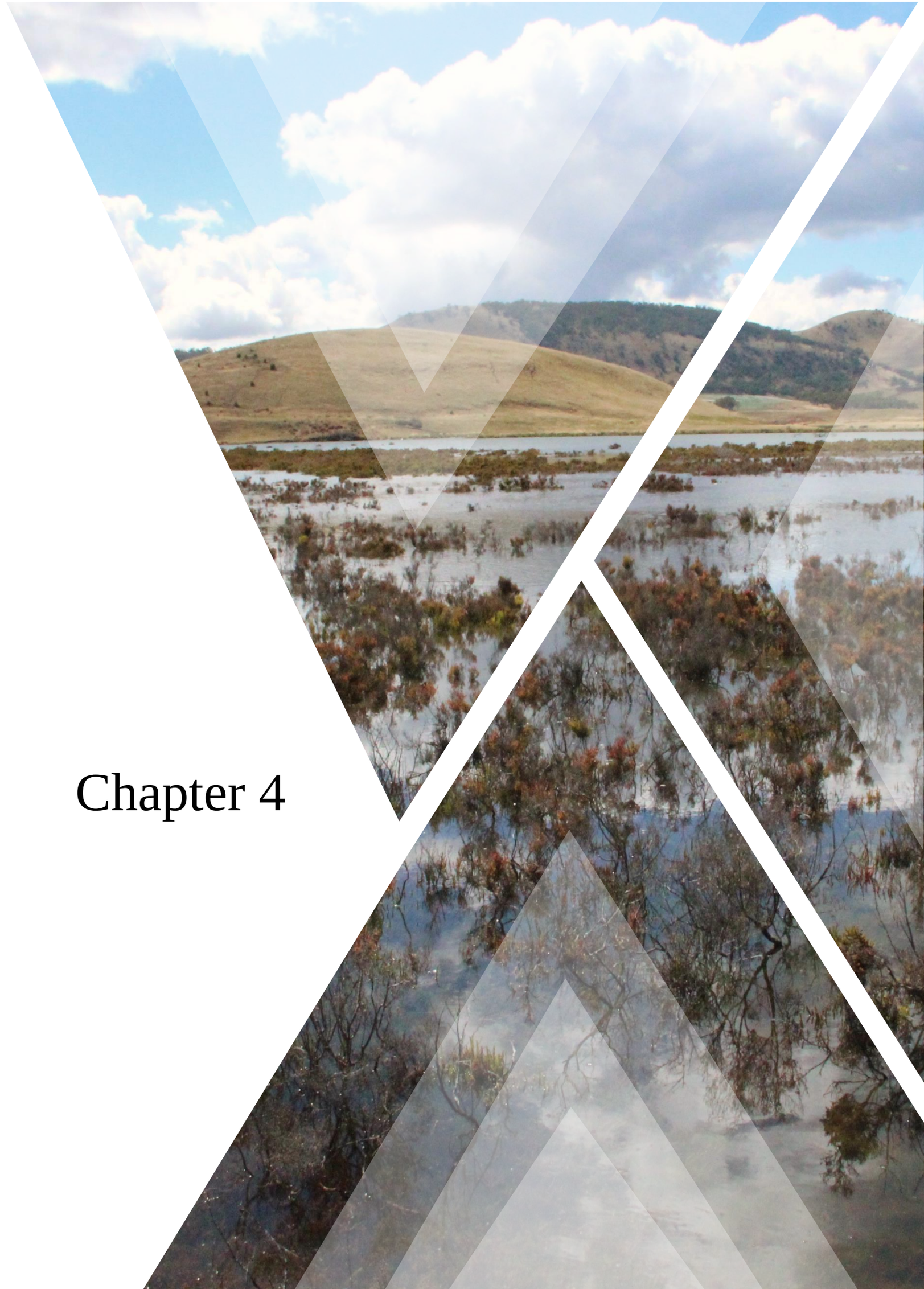
Increased samphire cover was the only shared predictor amongst sites for mosquito abundance. Previous studies on vector distribution within saltmarshes have shown a strong preference for regions with samphire, (Gislason and Russell 1997, Dale et al. 2008, Jacups et al. 2009, Rowbottom et al. 2017) as this habitat provides substrate for eggs of aedine mosquitoes. Other

well-known indicators of mosquito abundance include combinations of rainfall and high tides (Tong and Hu 2002, Kelly-Hope et al. 2004, Jacups et al. 2008, de Little et al. 2009, Werner et al. 2012, Koolhof et al. 2017), as these provide hatching triggers for *Aedes* mosquitoes (Bader and Williams 2011). In this study rainfall was absent from mosquito predictions, although we experienced lower than average monthly rainfall during our study period, particularly in the 2012-2013 season. Furthermore, the absence of tides as a predictor, particularly for first instars, is likely due to the inadequacy of the central tidal records to reflect the unique tidal relationship each marsh has in its location.

Saltmarshes are dynamic habitats, with constant changes in environmental conditions from temperature, rainfall and tide (Adam 2002). As such, the dynamic nature of these environments contributes to the variability within a saltmarsh (Carver et al. 2010b, Cadotte et al. 2011). At all sites we observed significant abiotic and biotic variability both within sites and between seasons. This variability contributed to the low mosquito predictability of our models. This was evident at the Primrose Sands site, which had the lowest mosquito abundance predictability and the highest variability in mosquito numbers and aquatic conditions of all three sites.

Here we have shown that seemingly similar saltmarshes can vary markedly in aquatic invertebrate FFGs owing to minor changes in water availability and conditions either through tidal inundations, rainfall or waterbody permanency. These aquatic differences were found to be important for seasonal persistence of a RRv vector mosquito. Our analysis also showed that the only constant predictor was samphire, which serves as oviposition habitat for the vector. Accordingly, our research has two essential implications; 1) availability of oviposition habitat should be a key environmental feature when assessing potential vector habitats and the vectorial risk of disease transmission; and 2) rich opportunities exist for research into the subtle differences characterising seemingly similar saltmarsh habitats, over a greater number than in the present study, to resolve the most important environmental drivers of aquatic invertebrate

FFG composition and mosquito disease risk. Our work has contributed knowledge that will help inform modelling and management of RRv and highlighted the importance of biodiversity conservation for the benefit of protecting public health.



Chapter 4

Chapter 4 – Mosquito distribution in a saltmarsh: determinants of eggs in a variable environment

Raylea Rowbottom* [a], Scott Carver [b], Leon A Barmuta [b], Philip Weinstein [c], Geoff R Allen [a]

[a] School of Land and Food/TIA, University of Tasmania, Hobart, Australia

[b] School of Biological Sciences, University of Tasmania, Hobart, Australia

[c] School of Biological Science, University of Adelaide, Adelaide, Australia

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A copy of the published article is provided in Appendix II.

Author contributions: RR, GA, SC, and LB conceived the project. RR and SC conducted the field work. RR carried out the laboratory work. RR SC and LB performed the statistical analysis. GA oversaw the project and provided conceptual guidance. RR wrote the manuscript with critical feedback from all authors.

4.0 Abstract

Two saltmarsh mosquitoes dominate the transmission of Ross River virus (RRv, Togoviridae: Alphavirus), one of Australia's most prominent mosquito-borne diseases. Ecologically, saltmarshes vary in their structure, including habitat types, hydrological regimes, and diversity of aquatic fauna, all which drive mosquito oviposition behavior. Understanding the distribution of vector mosquitoes within saltmarshes can inform early warning systems, surveillance, and management of vector populations. The aim of this study was to identify the distribution of *Ae. camptorhynchus*, a known vector for RRv, across a saltmarsh and investigate the influence that other invertebrate assemblages might have on *Ae. camptorhynchus* egg dispersal. We demonstrate that vegetation is a strong indicator for *Ae. camptorhynchus* egg distribution, and this was not correlated with elevation or other invertebrates located at this saltmarsh. Also, habitats within this marsh are less frequently inundated, resulting in dryer conditions. We conclude that this information can be applied in vector surveillance and monitoring of temperate saltmarsh environments and provides a baseline for future investigations into understanding mosquito vector habitat requirements.

Keywords

Ross River virus, *Aedes camptorhynchus,* oviposition, saltmarsh, *Sarcocornia,* ostracods

4.1 Introduction

Saltmarsh mosquitoes include major vectors of disease worldwide. This is particularly notable across the continent of Australia, where two saltmarsh mosquito species dominate transmission of Ross River virus (RRv), Australia's most prominent mosquito-borne disease. Accordingly, these habitats are a focus of major vector control and surveillance efforts targeting egg and larval stages of mosquito development. However, there is little knowledge about egg distribution of target mosquitoes within the complexity of saltmarsh environments. This knowledge is informative for determining potentially preferred oviposition habitats or areas of optimal egg survival, and thus can support control efforts, supplement early warning systems, and assist in more effective surveillance and management of vector mosquito populations. It can also contribute fundamental information on the natural history of this ecologically important environment.

Saltmarshes vary in their ecological structure, including habitat types, hydrological regimes, and diversity of aquatic fauna, all of which drive mosquito oviposition behavior (Adam 2002, McCall and Pennings 2012). Across Australia, saltmarsh vegetation is unique in the overlap of mangroves and halophytic succulent plants, grasses, and sedges between the tropics and cooler temperate regions, with mangroves being absent from Tasmania (Laegdsgaard 2006, Saintilan and Rogers 2013). This provides a mosaic of habitats and environmental conditions that can be utilized by mosquito vectors. Females of aedine mosquito species oviposit eggs on damp substrate or regions of the saltmarsh where water periodically occurs from tides or rainfall (Dale and Knight 2008). For example, several studies have associated *Aedes vigilax* (Skuse) with oviposition regions near drainage channels that are subject to tidal inundations (Gislason and Russell 1997, Jacups et al. 2009). Similarly, aquatic interactions between mosquitoes and predator/competitors within saltmarshes can also impact mosquito oviposition, larval development, survival, adult size (Mokany and Shine 2003b, Knight et al. 2004, Vonesh and

Blaustein 2010), and potentially egg survival. Consequently, these effects have potential to change mosquito fitness and vector capacity (Alto et al. 2005, Bara et al. 2015).

In southern Tasmania, *Ae. camptorhynchus* (Thomson) (Diptera: Culicidae) is a recognized vector of RRv (Harley et al. 2001, Russell 2002, Cutcher et al. 2017). This halotolerant species is the dominant mosquito in saltmarsh habitats and notoriously bites humans (Barton et al. 2004, Williams et al. 2009), with populations in southern Tasmania most abundant from Spring to Autumn (October-April) (Werner et al. 2012). The presence of other fauna (microcrustaceans) was formally proposed to have a role in regulating *Ae. camptorhynchus* abundance within saltmarshes (Carver et al. 2011). However, more recent laboratory evidence suggests this form of competition may have limited effects on *Ae. camptorhynchus* development (Rowbottom et al. 2015). Currently, little is understood about the distribution of *Ae. camptorhynchus* or its potential competitors within the saltmarsh.

At present, larval abundance of *Ae. camptorhynchus* is known to be associated with samphire (*Sarcocornia* sp.) habitats (Carver et al. 2011). Similar affiliations with samphire (*Sarcocornia quinqueflora* (Scott)) and salt-water couch (*Sporobolus virginicus* (Kunth)) have been identified for populations of *Ae. vigilax*, an ecologically similar species, although more prevalent in warmer Australian climates (Dale et al. 2008). However, other environmental conditions, such as hydrology, and/or competition with other aquatic fauna can also alter mosquito distribution. Therefore, the aims of this study were to (1) determine saltmarsh habitats in which *Ae. camptorhynchus* eggs are most abundant, (2) investigate environmental conditions that may favour mosquito abundance within saltmarsh habitats, and (3) examine the use of the same sites by ostracods, the dominant microcrustacean species and putative competitor of *Ae. camptorhynchus* in this saltmarsh, and other invertebrates.

Given the spatial occurrence of both *Ae. vigilax* and *Ae. camptorhynchus* throughout mainland Australia, we expect to observe greater egg distribution among samphire (*Sarcocornia* sp.). We also expect that hydrological influences, such as tidal inundations and heavy rainfall, would affect egg dispersal and abundance. Considering the absence of significant predator species in this saltmarsh, we do not expect the presence of other invertebrates to influence egg distribution, but given the dynamic nature of saltmarsh habitats, we discuss other mechanisms that may have a role in egg distribution.

4.2 Methods

4.2.1 Field sampling

This study was conducted at a saltmarsh in Primrose Sands (42.87° S, 147.65° E) in southern Tasmania (Figure 4.1). This saltmarsh is known for its seasonally large populations of *Ae. camptorhynchus* (Robertson et al. 2004, Carver et al. 2011). Overall, this marsh ranges in elevation from 0.38 m to 3.2 m with a bank that reaches above 1.5 m along the Carlton River, a saline and tidal area of the river near its terminus with the ocean. Cores of vegetation and soil were collected from the saltmarsh to evaluate egg distribution of *Ae. camptorhynchus* and other invertebrates, including dormant microcrustaceans. We sampled the saltmarsh by categorizing the environment into four dominant habitat types; 1) bare soil (BS), 2) runnels (human-made from early 1950s) (R), 3) samphire/beaded glasswort (*Sarcocornia quinqueflora*) (S), and 4) shrubby glasswort (*Tecticornia arbuscula* (Sheph and Wilson)) (SG). The saltmarsh was longitudinally divided into three 1 km sections that allowed for sampling from the river side of the marsh, centre marsh, and the embankment. Each transect was separated by 47 m and followed a bearing of 030 along the main edge of the Carlton River. To make sure each habitat type was sampled evenly across the saltmarsh, all three transects were divided into 200 m

quarters in which all four dominant habitat types were sampled (Figure 4.1). Field samples were collected over the peak season of RRv and mosquito abundance in Tasmania (October-March) at times when an abundance of larvae and pupae were observed. These collections also coincided with an absence of excessive rainfall and tidal inundations for approximately one week to allow enough time for adult emergence, mating, feeding, and oviposition to occur (Gillett 1971).

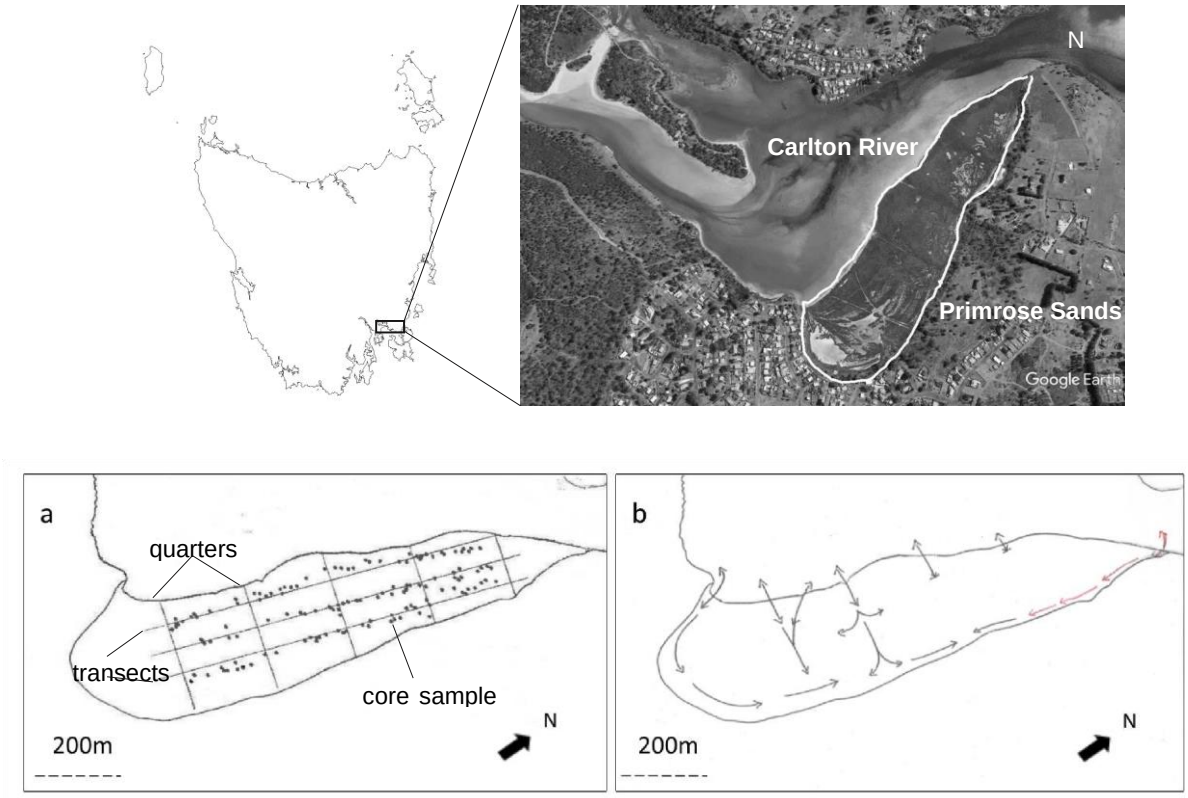

Figure 4.1. A map of the study area in southern Tasmania, displaying the saltmarsh region of Primrose Sands along the Carlton River. Sampling design including transect lines and core locations are presented in (a), and tidal hydrology (b). Detailed images of the saltmarsh are tilted to the east for aesthetics.

There were three sampling periods, October, December, and February 2012-2013. To maintain random sampling of habitat types within each quarter of each transect, a random starting point (distance in meters from the beginning of each transect) was allocated for each transect. From the start of each transect, increments of 10 m were travelled at which point a core sample was taken, providing there was a suitable un-sampled habitat type. If not, the distance was travelled again until all four habitat types were sampled within each quarter. At each consecutive sampling period, December and February, the starting point of sampling from each transect line was taken at 6 m and 32 m, respectively. This was to ensure core samples were not from the same locations as the previous sampling periods. Stainless steel circular cores (589 cm^3) were used for all core samples. Once extracted, each core was placed in 20 cm round plastic containers for transport and laboratory inundations. The drainage systems, due to their positioning in the marsh and possibility of being un-sampled in the above system, were individually targeted within each quarter.

At each sampling location, for each habitat type, a GPS location was recorded as well as temperature using a hand-held logger. In addition, the soil water content was measured for each habitat type for each transect by taking a 141 cm^3 soil core sample. These were placed in a 21 x 15 cm zip-lock bag and the wet weight recorded. To assess the dry weight of these soil cores, each sample was removed from the zip lock bags and placed in 25 x 14 cm foil trays and dried in an oven (Qualtex 68 R4) at 105°C for approximately two weeks. At the completion of the drying period all cores were placed back in their zip-lock bags and re-weighed.

4.2.2 Environmental data

Rainfall, temperature, and tide height were all recorded over the collection period. Daily maximum and minimum temperature were recorded using temperature loggers (Thermocron TC, On Solution Pty. Ltd) set to record every hour. These were positioned at the saltmarsh 1 m

above the ground in Stevenson screens and data were extracted monthly. Rainfall was measured from rain gauges situated in the centre of the marsh that were emptied weekly. Tidal height was obtained from titanium HOBO water level loggers (Onset Solutions, U20-001-04 Ti) placed in stilling wells. Stilling wells consisted of 25 cm long, 8 cm diameter PVC pipes with sealed caps on either end. Seven horizontal incisions were cut at the base of the pipes to allow for water flow/drainage. Inside the pipes sat the titanium HOBO water level loggers which were encased in PVC tube sitting on a bolt. Each stilling well was attached to a star picket to prevent damage and allow accuracy of readings.

4.2.3 Laboratory conditions

To assess which habitat type was most likely for *Ae. camptorhynchus* egg distribution, the top 10 cm of soil from each core was cut from each sample and placed back into the plastic containers (along with any plant material). All cores were kept at 10°C for 48 h then placed in a temperature controlled unit (Andrew Thorn Limited Qualtex 68 R4) set to 20 ± 0.3°C 12:12 day:night cycle, and inundated with 250 ml saline water at 35 ppt ("Red Sea Salts"; at 35 ppt elements are: 8.2-8.4 pH, 7.8-8.3 Alk (dKH), 420440 Ca (mg/liter), 1250-1310 Mg (mg/liter), and 380-400 K (mg/liter)). Cores were inundated to promote hatching of dormant eggs and to examine the species assemblage of the different habitat types as a means of assessing the relationship between mosquito and macroinvertebrate egg distribution (Banerjee et al. 2010). The temperature chosen for laboratory conditions was based on the average spring/summer daily temperatures of water bodies in the field near Hobart (Rowbottom, *unpublished data*).

All cores were monitored daily to make sure they remained submerged, with water being replaced *ad-libitum*. To prevent the water going stagnant, a 19 gauge 1½ inch hypodermic needle (Neolus Terumo needles 1938) connected to one of two Resun AC4000 Air Pumps using 8 mm plastic PVC hosing was placed inside each soil core container. All cores remained

wet for five days at which time all the water was strained through a 150-micron sieve (99 µm) so that invertebrate emergence could be scored. The core was then placed back in the temperature cabinet and left dry for seven days prior to a second inundation following the same procedure. Two wetting cycles were used to simulate environmental inundations from rainfall or tide and to allow for instalment hatching (Bader and Williams 2011). All cores were randomly placed within the cabinet for both wetting cycles. All culicids were identified to species, with other invertebrates identified to order.

4.2.4 Data analysis

To identify *Ae. camptorhynchus* egg distribution, we evaluated how extrinsic components of the saltmarsh affected mosquito abundance. The bayesian model, described below, was the preferred analysis for all response variables because it is able to determine coefficients in mixed models with more accuracy (Gelman and Hill 2006) and the Poisson error distribution was most appropriate to handle over-dispersion given the use of count data.

Mosquito egg distribution was evaluated using Bayesian regression with habitat and elevation as the fixed effects, with month as a linear and quadratic term. Y_i was the average number of emerged mosquitoes within each replicate $i = 1,\ldots,n_i$. The distribution of eggs among replicates was assumed to have a Poisson distribution with parameter π_i:

$$Yi|\pi i \sim \text{Poisson}\ (\pi i)$$

where π_i is the modelled egg distribution of replicate i. We modelled the egg density, π_i, based on the effects of environmental variables,

$$\pi i = \alpha i + \beta i x i$$

where α and β are the model intercept and slope, respectively, for replicate *i* and x was the assigned environmental variable for replicate *i*. Prior distributions for all model parameters in the hierarchy were given with the goal of providing conjugate priors that contain little to no influence on the posterior distributions of all the model parameters. We assumed normal prior distributions on slopes, α, and intercepts, β, with mean μ and variance σ^2:

$$\alpha \sim \text{Normal}(\mu_{\alpha}, \sigma^2_{\alpha}) \; \beta \sim \text{Normal}(\mu_{\beta}, \sigma^2_{\beta})$$

For the variance parameters, σ^2, we determined and utilized non-informative uniform prior hyperparameter distributions, specified as $\sigma^2 \sim$Uniform (0, 100), which was used across all models. Models were fitted in R (v 3.0.3) (R Core Team 2019) using the 'MCMCglmm' package, with MCMC chains run for 13,000 iterations after a burn-in period of 1,000 iterations, ensuring convergence of model parameters, assessed following Gelman and Hill (2006). We summarized posterior distributions of model coefficients, β, by the Bayesian median and 95% credible intervals and MCMC simulated *p*-values. The effect of extrinsic variables on ostracod density and other invertebrate assemblage was also assessed using the above method.

4.3 Results

Sampling covered the primary months when mosquito presence was expected to be observed in southern Tasmania. During this time, weather conditions remained constant with monthly daily maximum temperatures averaging 33.4°C (Table 4.1). There were no tidal events that resulted in the inundation of the entire saltmarsh, with the highest recorded tide reaching 1.84 m (Table 4.1).

Table 4.1. In situ weather data for the Primrose Sands saltmarsh, Tasmania, over the collection period (October 2012–February 2013). Rainfall, temperature, and tide height data include monthly ranges and averages ± SD. Missing data are due to local bushfires.

Variable	Oct	Nov	Dec	Jan	Feb	Overall Range
Rainfall (mm)	0 – 12	0 – 21	0 – 6	0 – 2.5	0 – 6	0 – 21
Mean ± SD	4.25 ± 5.32	6.4 ± 9.45	2.5 ± 3	0.5 ± 1.12	3.33 ± 3.06	4.21 ± 5.41
Temperature (°C)	3 – 30	5 – 33	5.5 – 33	0	6.5 – 35.5	3 – 35.5
Mean ± SD	13.52 ± 7.04	16.68 ± 7.46	11.88 ± 11.58	0	13.70 ± 11.25	12.21 ± 10.05
Tide (m)	0.71 – 1.64	0.72 – 1.62	0.65 – 1.78	0.84 – 1.84	0.83 – 1.70	0.64 – 1.84
Mean ± SD	1.01 ± 0.21	0.97 ± 0.21	1.06 ± 0.25	1.09 ± 0.26	1.00 ± 0.21	1.03 ± 0.23

The water content of soil cores varied between habitat types of the Primrose saltmarsh (26% to 55%; samphire and runnels, respectively). Runnels had the greatest water content followed by bare soil with samphire and shrubby glasswort having the least water content (Figure 4.2 and Table 4.2). There was no association with water content and elevation. The water content of soil samples increased with each consecutive month of sampling (Table 4.2).

A total of 12,265 invertebrates emerged and was counted from the soil cores throughout the study period; of these, 3.6% (438) were *Ae. camptorhynchus* and 7.7% (947) were ostracods. A large constituent of the remaining invertebrates was a ubiquitous Copepoda (Table 4.3) with 79% (9688) individuals identified, however, both copepods and other invertebrates did not influence the distribution of *Ae camptorhynchus* eggs (Table 4.4).

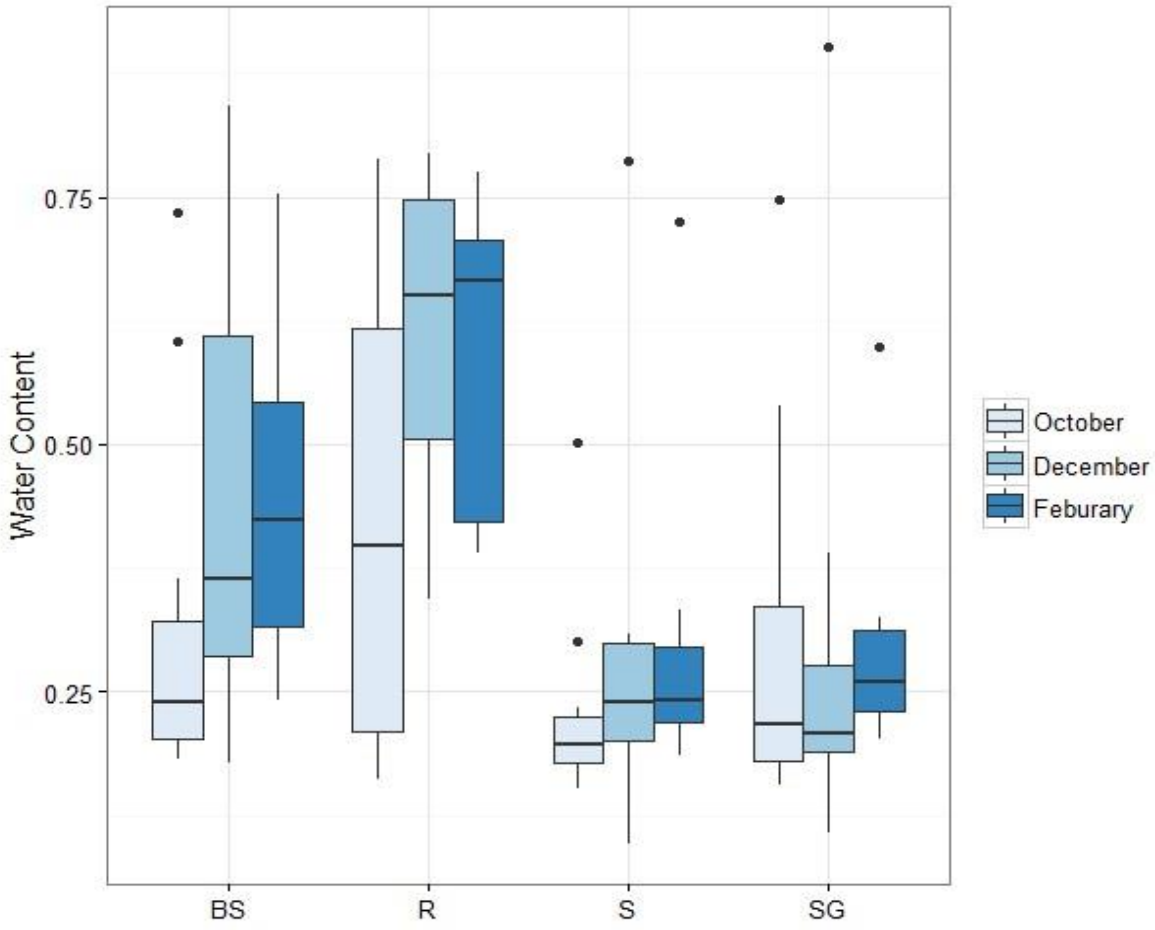

Figure 4.2: Percent water content from each habitat type for the three months of collection. Habitat types are as follows; (BS) bare soil, (R) runnel, (S) samphire and (SG) shrubby glasswort.

Table 4.2: Bayesian mixed-effects regression results showing the differences in water content for habitat type (bare soil, runnel, shrubby glasswort, samphire), elevation, and month of collection. Variables with significantly different water content are in bold. Credible intervals are 95%.

Variable	Coeff.	2.5% CI	97.5% CI	pMCMC
(intercept)	-0.716	-1.326	-0.097	**0.030**
Runnel	0.318	0.118	0.513	**0.004**
Samphire	-0.400	-0.613	-0.213	**<0.001**
Shrubby glasswort	-0.320	-0.506	-0.124	**0.006**
Elevation	-0.397	-1.125	0.380	0.326
Month.L	0.197	0.078	0.313	**<0.001**
Month.Q	-0.061	-0.193	0.055	0.312

L - linear and Q - quadratic.

Table 4.3: A complete list of the remaining taxa identified from each habitat type in the core samples from the collection months.

	Copepods	Diptera	Gastropod	Collembola	Coleoptra	Acari	Amphipod	Oligochaetes	Unidentified
October									
Bare soil	1,509	9	0	0	6	4	1	0	2
Runnel	1,838	16	4	0	0	1	0	6	1
Shrubby glasswort	1,177	42	3	1	9	15	1	66	7
Samphire	2,527	78	1	2	3	29	0	47	29
December									
Bare soil	183	5	0	3	1	15	0	2	0
Runnel	315	30	1	1	0	10	0	0	0
Shrubby glasswort	367	4	0	11	6	32	1	32	0
Samphire	327	70	1	2	1	32	0	95	2
February									
Bare soil	57	6	0	0	1	2	0	3	0
Runnel	193	14	0	0	1	2	0	1	1
Shrubby glasswort	784	78	0	1	4	9	3	82	12
Samphire	411	106	2	0	9	23	0	78	14

Table 4.4: Bayesian negative binomial results showing the effects of other invertebrates on *Ae. camptorhynchus* egg distribution. None of the variables contributed to *Ae*. *camptorhynchus* densities. Credible intervals are 95%.

Variable	Coeff.	2.5% CI	97.5% CI	pMCMC
Copepoda	-0.006	-0.023	0.007	0.408
Chironomidae	0.132	-0.155	0.394	0.318
Amphipoda	-2.442	-8.531	3.175	0.396
Acari	0.257	-0.296	0.764	0.334
Coleoptera	0.660	-1.626	2.763	0.532
Collembola	-3.317	-7.764	0.505	0.090
Diptera	0.470	-0.453	1.390	0.304
Gastropoda	-2.939	-9.895	2.189	0.294
unidentified	0.364	-0.269	0.997	0.258

Mosquitoes identified from the samples were exclusively *Ae. camptorhynchus* larvae. Habitat explained more variance in the *Ae. camptorhynchus* egg distribution than any other tested variable with 67.1% of mosquitoes emerging from cores collected from samphire habitats (Figure 4.3 and Table 4.5). Of the remaining habitats, 29.9% emerged from shrubby glasswort cores with only 1.4% and 1.6% from bare soil and runnels, respectively. In comparison, ostracod numbers dominated the bare soil and runnel samples with a total of 82.3%. Shrubby glasswort and samphire habitats consisted of only 5.9% and 11.8% of ostracods, respectively. However, both the sporadic distribution and extreme fluctuations of ostracods resulted in no significant habitat preference (Figure 4.3 and Table 4.5). Despite the dominant habitat distribution of both ostracods and *Ae. camptorhynchus* being dissimilar, increases densities of these arthropods were from neighbouring sites within the marsh (Figure 4.4). There was no significant association between ostracod abundance and *Ae. camptorhynchus* abundance (Table 4.5). There was little evidence that month affected the number of mosquito eggs present (Table 4.5). Apparent temporal increases in mosquito numbers (51, 116, 271 mosquitoes for October, December, and February respectively; Figure 4.3) were, also, driven by high egg numbers in a small number of samples with observed mosquito numbers ranging between 1 and 132 from a single core. By comparison, ostracod numbers peaked earlier in the sampling season with a maximum of 369 ostracods observed in bare soil samples in October and 296 in December. By February, numbers of ostracods fell below 75 individuals per habitat type. However, month did not significantly affect ostracod densities (Figure 4.3 and Table 4.5). The range of ostracods observed from a single core sample ranged between 1 and 230.

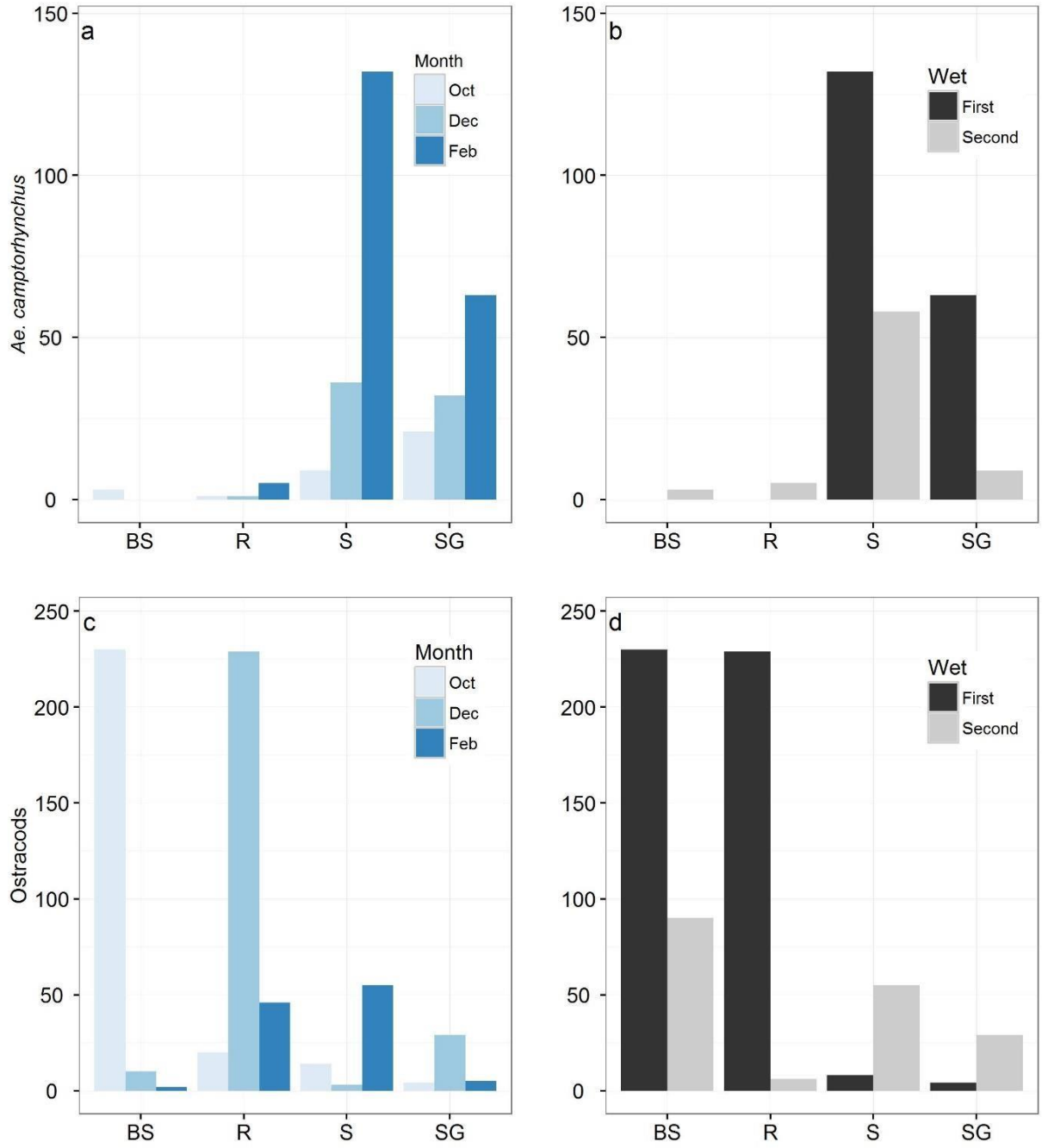

Figure 4.3: Total emergence *Ae. camptorhynchus* larva (a) and ostracods (c) from core samples for the three months of collection and total *Ae. camptorhynchus* (b) and ostracods (d) emergence from wetting cycles. Habitat types are as follows; (BS) bare soil, (R) runnel, (S) samphire and (SG) shrubby glasswort.

Table 4.5: Bayesian negative binomial results showing the effects of habitat type (bare soil, runnel, shrubby glasswort, samphire), elevation, month of collection and density of ostracods on *Ae. camptorhynchus* egg distribution. Effects of ostracod densities with *Ae. camptorhynchus* abundance is shown as the final variable. Variables that contributed to *Ae. camptorhynchus* or ostracod densities are in bold. Credible intervals are 95%.

Variable	Coeff.	2.5% CI	97.5% CI	pMCMC	Coeff.	2.5% CI	97.5% CI	pMCMC
		Ae. camptorhynchus				Ostracods		
Runnel	-0.037	-5.222	4.604	0.982	0.107	-1.851	2.058	0.914
Samphire	4.801	0.931	9.488	**0.012**	-0.936	-2.877	1.051	0.356
Shrubby glasswort	1.882	-2.488	5.993	0.390	-0.940	-3.103	0.964	0.328
Elevation	4.112	-11.980	20.695	0.590	4.195	-3.773	12.425	0.312
Month. L	-1.230	-3.592	0.986	0.284	-1.098	-2.497	0.107	0.094
Month. Q	0.472	-2.023	2.844	0.732	-0.744	-1.954	0.476	0.248
Ostracods	-0.047	-0.162	0.030	0.330				

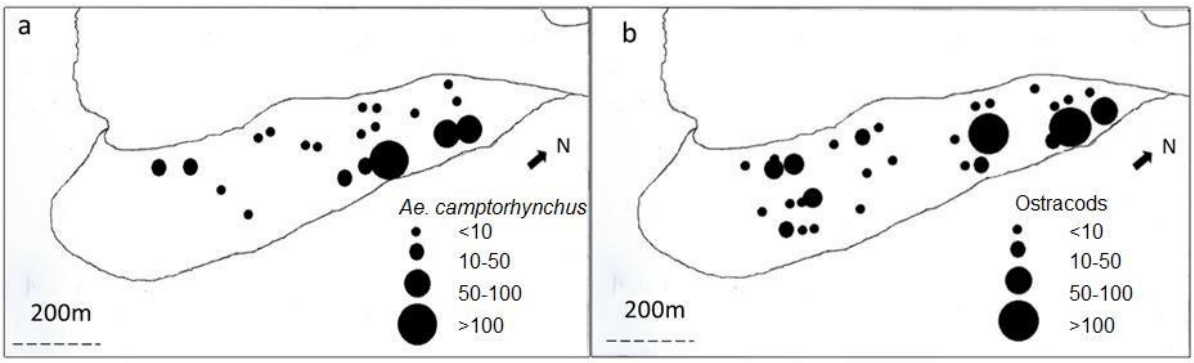

Figure 4.4: Collective density and distribution of *Ae. camptorhynchus* (a) and ostracods (b) from cores collected over the study period.

4.4 Discussion

Where a mosquito deposits eggs is an essential element in its life history, shaping individual fitness, development, and vectorial capacity (Bentley and Day 1989, Stone et al. 2012). Because of this, research into understanding the drivers that influence mosquito egg dispersal, especially of vector mosquitoes, is essential for mosquito management, control, and evaluating disease risk. However, globally these habitats are extremely variable both environmentally and ecologically (Laegdsgaard 2006), thus resulting in variability of mosquito egg distribution. For example, populations of *Ae. taeniorhynchus* in Florida are driven by substrate moisture and frequency of tidal inundations (Knight and Baker 1962, Ritchie and Addison 1992), whereas in Argentina, separate environmental factors drove abundance of two mosquito species in relation to their ovipositional strategies (Cardo et al. 2012).

Notably, the Australian coastline spans several degrees of latitude with saltmarsh and mangrove habitats being ecologically variable from the northern, tropical regions to the southern-most temperate regions (Adam 2002). Mosquito research in Australia has focused on vector mosquitoes that utilize these coastal saline environments as they produce the majority of endemic vector mosquitoes (Russell 1993, Russell 1995, Dale et al. 2008). When

determining mosquito egg dispersal and oviposition locations within these environments, it may be difficult to disentangle oviposition site preferences from potential field predation and egg survival. However, *Aedes* species display less predator avoidance with oviposition which has been proposed to be due to their egg-laying strategy and egg longevity in the field (Bentley and Day 1989, Vonesh and Blaustein 2010). Given the variability in mosquito oviposition and environmental conditions, local studies of saltmarsh habitats and the mosquito vectors within these environments contribute to current and future vector management by explaining knowledge of the most productive habitat and environmental conditions for vectors to emerge from, and thereby target, for control.

We conducted preliminary investigations of a southern Tasmanian saltmarsh that is a known region of large populations of *Ae. camptorhynchus* (Robertson et al. 2004). This mosquito is a prominent vector for RRv in Australia and the only vector for the virus in Tasmania (McManus and Marshall 1986, McManus 1994). Several studies have identified environmental drivers of *Ae. camptorhynchus* populations (Dhileepan et al. 1997, Barton et al. 2004, Werner et al. 2012), but there are no empirical data on egg distribution or site selection cues. Therefore, understanding the dynamics of this saltmarsh and the components that drive egg distribution of *Ae. camptorhynchus* will be useful in the management, surveillance, and implementation of any future targeted mosquito control (Webb and Russell 1999, Griffin et al. 2010, Kay and Russell 2013).

We determined samphire (*S. quinqueflora*) to be a dominant habitat type for *Ae. camptorhynchus* egg distribution in the Primrose saltmarsh, which is consistent with our understanding of aedine mosquito oviposition (Sinclair 1976). In Australia, a large proportion of research for vectors of disease in saltmarshes has focused on *Ae. vigilax*, with vegetation, in particular *Sarcocornia* spp., being the dominant determinant of oviposition sites (Dale et al. 1986, Gislason and Russell 1997, Dale et al. 2008). Yet in this study, the distribution of *Ae.*

camptorhynchus was not as extensive as the distribution of *S. quinqueflora*, suggesting that not all regions occupied by this habitat are suitable. Saltmarsh vegetation is characterized by the tidal frequency, salinity, and the influence of surrounding land use (Saintilan 2009a). Changes to these conditions, such as increases in sea level or increased nutrient input from surrounding regions, can drastically influence floral composition (Prahalad et al. 2012) and therefore mosquito presence. Thus, both soil moisture and elevation may be relevant factors contributing to egg distribution.

Generally, there are strong correlations among elevation, tide height, and soil moisture within saltmarshes (Saintilan 2009b, Prahalad et al. 2012), which also correspond to mosquito abundance (Dale et al. 2008, Jacups et al. 2009). Moisture is an essential component in saltmarsh mosquito egg conditioning (Knight and Baker 1962, Kay and Jorgensen 1986) and hatching (Andreadis 1990, Rydzanicz et al. 2011, Cardo et al. 2012) and many studies correlate *Aedes* spp. with wet, lower regions of saltmarshes that are periodically flooded by tide (de Little et al. 2009, Jacups et al. 2009, Cardo et al. 2012). We found that soil moisture was greatest in bare soil and runnel habitat than shrubby glasswort and samphire, but there was no relationship between soil moisture and elevation. *Ae. camptorhynchus* egg distribution within the Primrose saltmarsh was greatest in samphire and unrelated to elevation, showing oviposition preference for habitat containing the least soil moisture. While this result appears counterintuitive, two non-mutually exclusive causes may explain this result: periodic inundation (flooding) of the saltmarsh by (1) very high tides and (2) heavy rainfall. The Primrose saltmarsh has a distinct bank, approximately 1.8 m along the river front of the marsh, which the tide would need to exceed to infiltrate the marsh. Thus, when flooding of the saltmarsh does occur it tends to be widespread. Similarly, Kokkinn et al. (2009) in South Australia report one of the factors determining abundance of *Ae. camptorhynchus* to be maximum tide heights, with peaks in adult abundance occurring only after such events. Given

the extent of egg viability for *Ae. camptorhynchus* (Bader and Williams 2011), and the stability of environmental conditions during this study, it may be advantageous for egg distribution of *Ae. camptorhynchus* to occur in normally dryer, more protected habitats in the saltmarsh to benefit survival and development.

Aside from habitat preference, another element in mosquito egg distribution is the presence of other invertebrates, namely, competitors and predators. Avoidance of both competitors and predators has been identified among mosquitoes, however, this behavior is not ubiquitous (Vonesh and Blaustein 2010). Therefore, we sought to understand the distribution and abundance of a putative competitor, a micro-crustacean ostracod (Carver et al. 2011). Significant inputs of water into these habitats are associated with mass hatching of eggs (Carver et al. 2011) at this site. During this study, we discovered that ostracods in this system are widely distributed across the saltmarsh with greater abundance in wetter regions but no differentiation in habitat type, which could be a result of recruitment by tide or shifts of egg banks by heavy rainfall or tidal inundation (Boulton and Lloyd 1992). In addition, there was no evident relationship between ostracod presence and mosquito distribution, suggesting that their significance as an aquatic competitor on mosquito egg distribution choices is minimal, and potentially ostracods and *Ae. camptorhynchus* may occupy separate niches within the water bodies (Rowbottom et al. 2015). Furthermore, there was no evidence that the presence of any other invertebrates found in these soil cores had any importance on *Ae. camptorhynchus* oviposition. The apparent lack of oviposition avoidance of *Ae. camptorhynchus* may also be a result of the ability to lay eggs above the water surface (Vonesh and Blaustein 2010, Yoshioka et al. 2012, Gonzalez et al. 2016) where they are protected from aquatic fauna that may consume them.

The results of our study demonstrate that vegetation are strong indicators for *Ae. camptorhynchus* egg distribution and that this is a dryer habitat within the saltmarsh. Also, it appears that *Ae. camptorhynchus* egg distribution is not influenced by elevation or other invertebrates, albeit a depauperate fauna, located at this saltmarsh. Given that this saltmarsh is surrounded by a singular uniform land type and that different surrounding land use has been associated with changes in mosquito productivity (Claflin and Webb 2017), it would be informative to compare mosquito abundance with other local saltmarshes with a diversity of surrounding land types.

Knowledge of egg distribution and vector emergence patterns has many applications for future management. At present, there is little management or surveillance of mosquito populations within Tasmania. In fact, there is little empirical data on the spatial distribution of this vector in the state (Lee 1949, Robertson et al. 2004). Considering that there are discrepancies with habitat types and egg densities within mangrove systems (Dale et al. 2014), and that oviposition preferences are highly complex, the information from this study will act as a guide for future mosquito surveillance and knowledge of habitats that are likely regions for mosquito vectors. This is particularly important given the projected increase in human proximity to saltmarsh and inland wetland regions where *Ae. camptorhynchus* populations are likely to be (Lyth and Holbrook 2015). Furthermore, current models used to predict outbreaks of RRv based on environmental data (Werner et al. 2012) can benefit from understanding the spatial distribution of vector eggs within saltmarsh habitats and how this translates to sea level rise and tidal inundations.

We conclude that although habitat preference of *Ae. camptorhynchus* may be like other *Aedes* spp., other environmental preferences such as soil moisture and tidal height are species specific and may vary in different spatial contexts. This study has management applications in that vector control efforts for *Ae. camptorhynchus* should consider samphire habitat, even at higher elevations in the saltmarsh and that mass saltmarsh inundation events may be the main driver of *Ae. camptorhynchus* abundance.

Chapter 5

Chapter 5 – Resource limitation, controphic ostracod density and larval mosquito development

Raylea Rowbottom* [a], Scott Carver [b], Leon A Barmuta [b], Dahlia Foo [b], Philip Weinstein [c], Geoff R Allen [a]

[a] School of Land and Food/TIA, University of Tasmania, Hobart, Australia

[b] School of Biological Sciences, University of Tasmania, Hobart, Australia

[c] School of Pharmacy and Medical Science, University of South Australia, Adelaide, Australia

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5.0 Abstract

Aquatic environments are subject to changes in both food resources and aquatic community structure as a result of competition and predation. Mosquito larvae, in particular, are sensitive to such changes resulting in altered developmental and adult traits. Resource limitation, predation and inter-, intra-specific competition within and between mosquito species are known to affect both mosquito development and survival, however, much less is understood about the effects of non-culicid controphic competitors (species that share the same trophic level). To address this knowledge gap, we investigated and compared mosquito larval development, survival and adult size in the presence of non-culidid controphic conditions and resource limiting conditions. We used *Aedes camptorhynchus,* a saltmarsh breeding mosquito and a prominent vector for Ross River virus in Australia. It generally lacks competitors due to its level of saline tolerance but does often co-occur with a sympatric ostracod micro-crustacean, which are found in dense populations in the same saltmarsh habitats. From our trials, resource limiting conditions caused significant effects by increasing mosquito developmental times and decreasing survival with significant decreases in adult size. However, context dependence aquatic conditions may be important in the similarity between observed adult traits amongst experiments as competition density did not affect larval development or survival. We conclude that the alterations of adult traits between non-culicid controphic competition may have impacts that extend to vector-borne disease transmission.

Keywords

Culicidae, Ostracod, *Diacypris* spp., developmental time, size, mosquito survival

5.1 Introduction

The effectiveness of competition for available food resources is driven by the presence of species within the same trophic level (controphic species) (Jennings and Kay 1999, Mitchell-Foster et al. 2012) that share the same resources and functional feeding group (Blaustein and Chase 2007, Juliano 2009). For mosquitoes, the presence of other species that are filter- and suspension-feeders can limit the amount of available food (Merritt et al. 1992). Consequently, the outcomes of controphic resource (exploitative) competition on mosquito development and survival should be equivalent to intraspecific increased resource limitation with greater effects on mosquito development, survival and adult size with increasing competition; however, this hypothesis is yet to be investigated.

Over the last two decades, significant effort has been directed to understanding the diverse effects that biotic interactions have on the ecology of mosquito vectors (Juliano 1998, Knight et al. 2004, Beketov and Liess 2007, Blaustein and Chase 2007, Werner et al. 2012). The larval life-stages of mosquitoes are the most sensitive to biotic interactions with impacts in developmental times, survival or changes to adult size, effecting fecundity and, for vectors of disease, vector competence capacity (Nasci and Mitchell 1994, Mokany and Shine 2003a, Schneider et al. 2011). To date, investigations have largely focused on larval predation, inter- and intra- specific mosquito competition (particularly with *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Say)) and mosquito oviposition behaviour as a means of predation and competition avoidance (Mokany and Shine 2003b, Eitam and Blaustein 2004). The importance of competitive interactions with other non-culicid invertebrates, particularly non-culicid controphic species, is poorly understood yet can play an important role in mosquito management (Mokany and Shine 2003a, Duquesne et al. 2011).

Across southern Australia *Ae. camptorhynchus* (Thomson) (Ballard and Marshall 1986, Russell 2002) is a major vector of Ross River virus (RRv: Togaviridae: *Alphavirus*). Epidemiologically, RRv is Australia's most important vector-borne disease with clinical notifications ranging between 1451–7754 per annum, resulting in an annual economic impact of approximately AU$10 million/year (Harley et al. 2001, Woodruff and Bambrick 2008). Saltmarshes are particularly important habitats for this halo-tolerant vector due to the hyper-saline aquatic conditions. Such physiologically extreme environments result in lower species diversity and hence fewer predators and competitors which is indicative of hyper-saline regions across Australia (Chapman et al. 1999, Russell 2002, van Schie et al. 2009, Carver et al. 2010b). *Ae. camptorhynchus* lays desiccation resistant eggs that undergo mass hatching with large pulses of rainfall or tidal inundations (Carver et al. 2009, Bader and Williams 2011). Likewise, these pulses of water also result in high densities of invertebrates such as micro-crustaceans that are capable of surviving dry periods (Williams 1997, Rossi et al. 2011). The *Diacypris* spp. (Crustacea: Ostracoda) that are prominent in these environments (*pers comm*, Walsh 2015) are detritovores/herbivores, occupying the same functional feeding group as *Ae. camptorhynchus*. Ostracods have also been implicated in decreasing available food resources for container-inhibiting mosquitoes (Merritt et al. 1992). Therefore, we need to understand how these interactions impact mosquito development. We expect that mosquitoes and ostracods compete for the same food resources within these habitats based on similarities in feeding and that these direct interactions will impact mosquito vector development, survival, abundance and size (Carver et al. 2011).

In this study we test the hypothesis that non-culicid controphic competition with ostracods reflects intra-specific resource limitation but with greater effects on mosquito development and survival with increasing controphic competition. We examined this using two experiments, an intra-specific resource limitation experiment with low densities of *Ae. camptorhynchus* and a

controphic resource limitation experiment with ostracods in increasing densities. Between the two experiments we contrast the changes in larval developmental times and survival, and effects on adult size. For both experiments, we predict decreased larval survival, increased development time, and reduced adult size as resources become limiting and controphic competition increases. We observed development and survival changes with intra-specific resource limitation and changes in adult size for both intra-specific resource limitation and controphic competition treatments. However, these changes are context dependant suggesting that the alterations of adult traits observed with non-culicid controphic competition may have impacts that extend to vector-borne disease transmission.

5.2 Materials and methods

5.2.1 Invertebrate collections

All mosquitoes and ostracods used in this study were sourced from the Primrose Sands saltmarsh (42.87° S, 147.65° E), east of Hobart, Tasmania, whereby permission to access the marsh was granted by the landowner. Water bodies found in the Primrose Sands saltmarsh are very ephemeral, lasting on average only 14 days after inundation over the peak of summer (Rowbottom et al. *unpublished data*). Such environments are very depauperate of aquatic species diversity as shown by Carver et al. (2011) where, after rainfall, 89% species diversity comprised of *Ae. camptorhynchus* (56%) and ostracods (33%), increasing to 91% in drying conditions (*Ae. camptorhynchus* 46% and ostracods 45%), a situation which could result in increased disease transmission (Keesing et al. 2010). As shown, this saltmarsh has only one abundant putative competitor of *Ae. camptorhynchus* (Carver et al. 2011), *Diacypris* spp. (Crustacea: Ostracoda) (*identified by*: Walsh 2015) and this was used in the competition experiment. Mosquitoes and ostracods were collected either after substantial rainfall or tidal

inundations that submerged most of the saltmarsh which provided newly hatched *Ae. camptorhynchus* larvae (1st instars, < 24 hours old). While every attempt was made to collect early first mosquito instars, age was difficult to control in practice, so for consistency examination of developmental rates in this study is restricted to the second instar onwards. All invertebrates were collected using a 350 mL plastic larval dipper (Australian Entomological Supplies Pty. Ltd.).

5.2.2 Laboratory conditions

Following field collections, mosquito larvae were placed into 500 mL translucent cylindrical plastic containers with 200 mL of water at 35 ppt salinity ("Red Sea Salts"; at 35 ppt elements are 8.2-8.4 pH, 7.8-8.3 Alk (dKH), 420-440 Ca (mg/L), 1250-1310 Mg (mg/L) and 380-400 K (mg/L)), with containers placed randomly in temperature cabinets (Andrew Thorn Limited Qualtex 68 R4) at 23°C ± 0.05 S.E., 14:10 day/night. The temperature and salinity were chosen for laboratory conditions based on the average summer daily temperatures (°C) and salinity (ppt) of water bodies in the field near Hobart (Rowbottom et al. *unpublished data*).

Invertebrate food consisted of ground "Nutrafin Max Fish Flakes" (Pets Domain). Four grams were ground using a mortar and pestle and suspended in 1 L distilled water. At each feed the solution was agitated to allow for homogeneity and refrigerated at 6°C between feeds to standardise the potential growth of microbes. Although not entirely analogous to their field based diets, food levels and type were representative of other laboratory studies of culicid nutrition and development (Jennings and Kay 1999, Tun-Lin et al. 2000, Farjana et al. 2012, Mitchell-Foster et al. 2012) and helped standardise nutritional quantity and quality which could otherwise vary if using field collected resources. New food was prepared fortnightly.

5.2.3 Experimental design

We conducted a paired experimental design to examine the effects of both intra-specific resource limitation and competition on mosquito development and survival. While a fully crossed design would have been optimal, this was beyond the scope of this study owing to the logistics of available incubators and number of ostracods required. Instead this study provides a paired design where intra-specific resource limitation and controphic resource limitation experiments are contrasted. In the resource limitation experiment 50 larvae were exposed to one of four food resource levels (0.1 mL, 0.2 mL, 0.4 mL or 0.6 mL food/day), with six replicate cylinders per treatment. For the competition trials 50 larvae were exposed to one of four treatments of competitor (ostracod) density (0 (control), 150, 300 and 600 ostracods/cylinder). This reflects the observed range of ostracod densities per 350 mL larval dippers (Australian Entomological Supplies) in water bodies at Primrose Sands between 2011 and 2012 (1-2144, n=442) (Rowbottom et al. *unpublished data*). Food resources remained at a constant level of 0.4 mL/day. Each treatment consisted of 10 replicate cylinders, with the competition control treatment being comparable to the 0.4 mL/day treatment in the resource experiment. Two replicates from each treatment from both experiments were dispersed evenly among three (resource experiment) or five (competition experiment) independent incubators (akin to blocks), with the position of replicates randomised within each incubator.

Daily counts of mosquito larvae included the number of larvae, instar of each larva, number of pupae and number and sex of adults. Any mosquito larvae or pupae that died were recorded and removed from the container. All emergent mosquitoes were collected, sexed and their wings removed and mounted using water onto glass slides and sealed with clear nail varnish. The average length of left and right wings were used as a proxy for adult size (Lounibos 1994). These were measured at 25× magnification from the wing tip (excluding the fringe) to the

arculus (Packer and Corbet 1989, Schneider et al. 2011) using Las EZ software (Leica Microsystems, Switzerland). Developmental time for instars – pupae in each replicate container was recorded as the day at which 50% of surviving larvae reached the next stage of development.

To account for size variation of ostracods amongst treatments, a subsample of five ostracods from each controphic resource limiting replicate was removed at the beginning of the experiment and again at the end of the experiment (when the last mosquito emerged or died in each replicate container). Measurements of the carapace, from posterior to anterior, were conducted using an ocular micrometer (0.016 mm units per graticule unit) on a Nikon SM2800 dissecting microscope at 6.3×magnification. All remaining ostracods in each replicate were scored for survival.

5.3 Analyses

In both experiments we evaluated how treatment affected larval developmental times, survival probabilities, and adult size of both sexes. The bayesian model, described for developmental times below, was the preferred analysis for all response variables, including the effect of competition treatment on ostracod mortality and size. as it is able to determine coefficients in mixed models with more accuracy (Gelman and Hill 2006).

Larval development time was evaluated using Bayesian mixed effects regression with treatment as the fixed effect and incubator as the random effect. Y_{ij} was the average developmental time when 50% of surviving larvae had reached the next instar or stage of development within each replicate cylinder $i = 1,\ldots, n_j$ for incubator $j = 1,\ldots, k$. The distribution of developmental times among replicates was assumed to have a Gaussian distribution with parameter π_{ij}:

$$Yij| \pi ij \sim \text{Gaussian}(\pi ij)$$

where π_{ij} is the modelled developmental time of replicate *i* in incubator *j*. We modelled the developmental time, π_{ij}, based on the effects of treatment

$$\pi ij = \alpha ji + \beta jixi$$

where α and β are the model intercept and slope, respectively, for replicate *i* varying by incubator *j*, and x was the assigned experimental treatment (intra-specific resource or controphic resource limiting level) for replicate *i*. Prior distributions for all model parameters in the hierarchy (incubators) were given with the goal of providing conjugate priors that contain little to no influence on the posterior distributions of all the model parameters. We assumed normal prior distributions on slopes, α, and intercepts, β, with mean μ and variance σ^2:

$$\alpha_j \sim \text{Normal}(\mu_\alpha, \sigma^2_\alpha), \text{ for } j = 1,\ldots,k$$

$$\beta_j \sim \text{Normal}(\mu_\beta, \sigma^2_\beta), \text{ for } j = 1,\ldots,k$$

For the variance parameters, σ^2, we determined and utilized non-informative uniform prior hyperparameter distributions, specified as σ^2~Uniform (0, 100), which was used across all models. Models were fitted in R (v 3.0.3) (R) using the 'MCMCglmm' package (Hadfield 2010), with MCMC chains run for 13,000 iterations after a burn-in period of 3,000 iterations, ensuring convergence of model parameters, assessed following Gelman and Hill (2006). We summarized posterior distributions of model coefficients, β, by the Bayesian median and 95% credible intervals and MCMC simulated *p*-values.

The effect of treatment on both survival and mean adult size (including effect of sex with wing length and treatment) were assessed using the above method. Survival over time could not consistently be identified at an individual level precluding assessment by Cox hazard models.

Therefore, we evaluated the effects of treatment on survival as total percent alive to adult for each replicate.

To assess consistency of results between experiments the 0.4 mL/day intra-specific resource limiting treatment and the controphic resource limiting treatment at the same food level without ostracods were compared for development, survival and adult size.

We also investigated ostracod size (measured through sub-samples) and mortality amongst competition treatments (end count of ostracods) to ensure that this was not a compounding factor to *Ae. camptorhynchus* developmental times, survival and size or ostracod development and mortality.

5.4 Results

5.4.1 Development time

Developmental time for *Ae. camptorhynchus* within each larval stage was increased through intra-specific resource limitation (Table 5.1). Developmental time from second through to fourth instar were significantly different amongst treatments with no effect of treatment on pupal developmental time. Mean developmental time to adult was 35 d with a range of 21-70 d for maximum developmental time among treatments (Figure 5.1; Supplementary III). In contrast, the time taken for *Ae. camptorhynchus* larvae to develop was only significant for third instar with all other instar and pupal stages having no significant affect by controphic resource limitation treatments (Table 5.1). Mean time to develop to adult was 27 d ranging between of 33-39 d maximum developmental time among treatments (Figure 5.1; Supplementary III). The overall developmental time between the 0.4mL/day intra-specific resource limitation treatment

and the controphic resource limiting treatment, without ostracods, at the same food level did not differ (p = 0.176) (Figure 5.1).

5.4.2 Survival

Aedes camptorhynchus survival decreased as intra-specific resource limitation increased (Table 5.1). The mean range of percent survival was between 61.4–96.1 (Figure 5.1; Supplementary III). By comparison, increasing competition did not affect *Ae. camptorhynchus* survival (Table 5.1), with a mean range of percent survival between 60.8–72.6 (Figure 5.1; Supplementary III). Survival between the 0.4mL/day intra-specific resource limitation treatment and the controphic resource limiting treatment, without ostracods, at the same food level was higher in the resource limitation than the competition experiments (p=0.028) (Figure 5.1).

Table 5.1. Bayesian mixed-effects regression results showing effects of both resource limitation and competition treatments on *Ae. camptorhynchus* larval development time (in days) between instar, pupae and total developmental time to adult, the effect of treatment on survival and the effect of both treatment and sex on adult wing length (mm) for both resource limitation and competition treatments. Significant *p* values are in bold.

Experiment	Resources				Competition			
Variables	coefficient	2.5%CI	97%CI	*p*	Coefficient	2.5%CI	97%CI	*p*
				Development (days)				
Larval Stages								
Second instar	-0.710	-1.169	-0.189	**0.006**	-4.74e-5	-2.15e-3	1.55e-3	0.984
Third instar	-3.379	-4.594	-1.789	**<0.001**	0.002	0.260-3	0.003	**0.008**
Fourth instar	-1.431	-2.405	-0.293	**0.004**	0.003	-0.002	0.008	0.262
Pupae	0.036	-0.137	0.207	0.654	0.002	-0.003	0.008	0.398
Overall	-6.893	-8.658	-5.205	**<0.001**	0.003	-0.007	0.013	0.55
				Survival				
Treatment	11.257	8.088	14.201	**<0.001**	0.006	-0.024	0.036	0.696
				Wing length (mm)				
Treatment	0.069	0.061	0.077	**<0.01**	<-0.001	<-0.001	<-0.001	**<0.01**
Sex	0.038	0.007	0.068	**0.02**	-0.093	-0.037	0.017	0.526

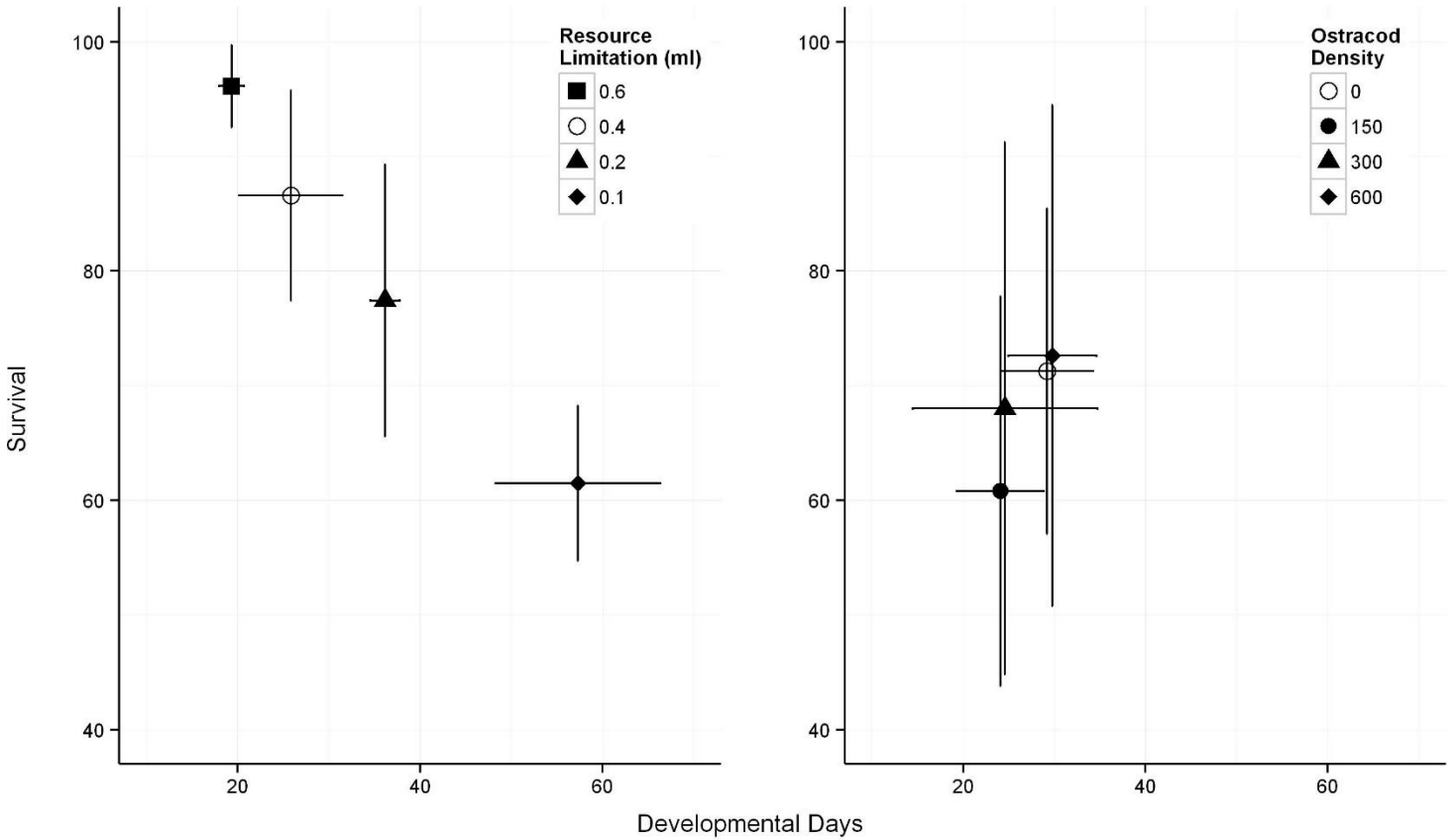


Figure 5.1: Effects of resource limitation (left panel) and competition (right panel) on *Ae. camptorhynchus* mean development time and survival to adult. Error bars represent the standard deviation. Treatments with open symbols are directly comparable.

5.4.3 Adult size

Size of *Ae. camptorhynchus* wing length declined as intra-specific resources became more limiting (Table 5.1). Overall, adults emerging from treatments with the most resources were 9.86% larger than all other resource treatments (Figure 5.2). There was also a sex-specific significance in size with males being 0.15 mm larger than females, however this was only in the most limiting resource treatment. Likewise, increased controphic resource limitation resulted in decreased size of *Ae. camptorhynchus* adults (Table 5.1). On average adults emerging from the control were 4% larger than adults from the highest ostracod density treatment (Figure 5.2). There was no significant sex-specific size difference between treatments (Table 5.2). Adult size in both the 0.4 mL food/day resource treatment and the competition treatment without competitors at the same food level did not differ (p=0.91, Figure 5.2).

5.4.4 Ostracod mortality and size

Ostracods within the controphic resource limitation experimental treatments averaged 0.842 mm in length with no significant difference in size over the duration of the experiment (p=0.624). Similarly, ostracod survival was not significantly different between experimental treatments (p=0.628), with an overall average 17% loss of ostracods.

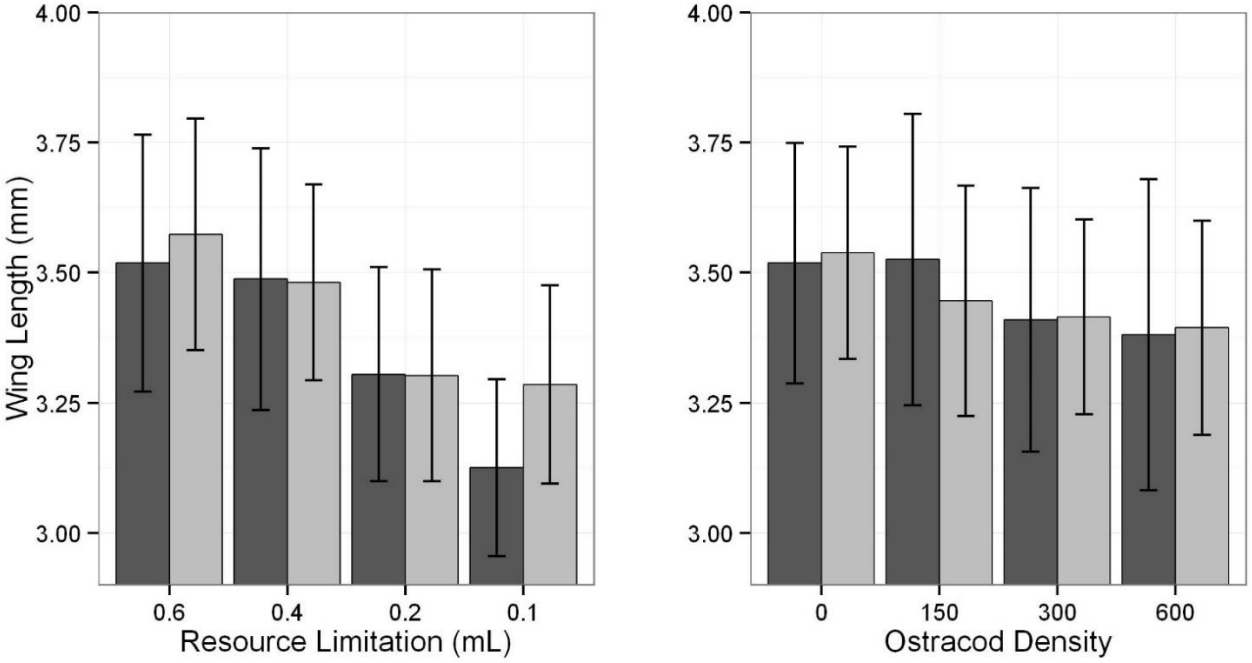


Figure 5.2: Mean wing length (mm) for *Ae. camptorhynchus* adult females (dark grey) and males (light grey) with standard deviation bars for both resource limitation (left panel) and competition (right panel) treatments.

5.5 Discussion

Like resource limitation, competitive interactions can affect mosquito life history thereby having an important role in the ecology of disease vectors. The results of our intra-specific resource limitation experiments are consistent with previous work that has investigated the effects of resource limitation on mosquito development and survival (Bevins 2007, Juliano 2007, Gilles et al. 2011, Padmanabha et al. 2011, Mitchell-Foster et al. 2012) showing increased developmental times, decreased survival and reduced adult sizes. However, this study also indicates that when numbers of ostracod controphic competitors are increased the intensity of the interactions are not significant enough to impact *Ae. camptorhynchus* developmental traits but do affect adult size. Adult body size decreased when controphic resource limitation with ostracods intensified reflecting, to a lesser degree, adult size effects observed with intra-specific resource limitation. Our study is the first to directly test non-culicid controphic competition with ostracods. We demonstrate that this interaction is an important component to mosquito ecology providing insight into the complexity of aquatic interactions with an important vector species.

As expected, increased intra-specific resource limitation drove increased developmental times for *Ae. camptorhynchus* (Jennings and Kay 1999, Bevins 2007, Gilles et al. 2011, Padmanabha et al. 2011, Mitchell-Foster et al. 2012) through intra-specific competition. In fact, in challenging resource conditions *Ae. camptorhynchus* extend their developmental time well beyond the mosquitoes within the controls (0.4 mL food/day treatment). Similar extension of developmental time has been demonstrated for both *Ae. albopictus* (Skuse) and *Ae. aegypti* in extremely limiting diets and this was inferred to indicate effective accumulation, assimilation and storage of energy gained from food resources (Barrera 1996). However, prolonged larval developmental times could have negative impacts in the field, especially in conditions where water bodies are extremely ephemeral such as saltmarshes. For example, extended

developmental durations in these aquatic habitats increase exposure to habitat loss through water bodies drying up (Knight 2011), thereby having the potential to result in decreased population sizes and reduced disease transmission.

Controphic competition is a relatively new field of research, with few studies detailing increased developmental times in the presence of non-culicid competitors (Knight et al. 2004, Stav et al. 2005, Blaustein and Chase 2007). In these situations, where resources are shared, it is common to expect exploitation competition of one species over the other, although this would normally result in the decline of one species (Morin 1999, Amarasekare 2003). It was expected that our non-culicid resource limiting treatments would negatively impact *Ae. camptorhynchus* development through ostracods utilizing greater resources (Merritt et al. 1992, Blaustein and Chase 2007, Rossi et al. 2011). Some examples include Duquesne et al. (2011) and Stav et al. (2005) where both authors presented decreased developmental times of *Culex pipens* by up to 5 days with *Daphnia magna* (Crustecea: Cladocera). Despite their use of greater numbers on controphic competitors than what we presented in our trials, the only significant decline in development we observed was during the III instar, with no effect of overall developmental times between treatments. While it is plausible that the densities within our trials were inadequate to result in exploitative competitive outcomes on *Ae. camptorhynchus* life history, the number of ostracods used in these trials reflect ostracod densities found in natural water bodies (Carver et al. 2011, Rowbottom, *unpublished data*). Therefore, given that *Ae. camptorhynchus* maintained development within the greatest density of ostracods and hence the most resource limiting treatment, and ostracod mortality was not significant across treatments, exploitative competition is unlikely to be the interaction in this situation.

Unlike our intra-specific resource limiting trials whereby *Ae. camptorhynchus* survival was significantly affected by increasing intra-specific resource competition, *Ae. camptorhynchus* survival did not change with increasing densities of ostracod competitors. Declines in mosquito

survival have been documented in other controphic competitive studies (Mokany and Shine 2003a, Blaustein and Chase 2007), for example, Mokany and Shine (2003a) demonstrated a decline in *Ae. australis* survival as a result of interference competition between tadpoles (*Limnodynastes peronii*), however this was a result of chemical interference which has not been documented within ostracods. In contrast, Daugherty and Juliano (2003) demonstrated improved survival of *Oc. triseriatus* in the presence of higher densities of scirtid beetles because of the quantities of faeces excreted by the scirtids and attached microorganisms that were consumed by the developing mosquito larvae. Although this is possible, we would have still expected to see some degree of change in survival or development amongst the competition treatments whereas no effect was observed. Nevertheless, this mechanism deserves further exploration.

Even though adult size was negatively affected by intra-specific resource limitation, males were significantly larger than females for the most limiting resource treatment. It is known that larval females can take longer to develop as a trade-off for greater accumulation of resources which ultimately results in larger sizes and improved fecundity (Clements 1992, Blackmore and Lord 2000). However, under increased non-culicid resource limitation, there may be an effect on female *Ae. camptorhynchus* reproductive success. In fact, resource limitation is correlated with fewer oogenesis cycles resulting in reduced fecundity (Telang et al. 2006) and a decreased ability of mosquitoes to carry and transmit disease (Nasci and Mitchell 1994, Jennings and Kay 1999, Muturi et al. 2011, Mitchell-Foster et al. 2012). Due to *Aedes camptorhynchus* being anautogenous (Barton and Aberton 2005) thereby requiring a blood meal to complete oogenesis, it is likely that this reduction in size for *Ae. camptorhynchus* females limits vectorial abilities, although further research is necessary to understand the connection with *Ae. camptorhynchus* size and the ability to transmit RRv. A potential caveat is that experimental adults would not represent field adults, however, we have found substantial

overlap in mean wing length between field caught adults and experimental adults (competition, 3.46 ± 0.27, resources, 3.37 ± 0.26 and field, 3.60 ± 0.44).

Similarly, a reduction in adult size was also observed with increasing ostracod competition a phenomenon that has been demonstrated in previous studies (Mokany and Shine 2003a, Stav et al. 2005), however, given the lack of developmental or survival changes in our trials with increasing ostracod densities our study indicates that it may be common for *Ae. camptorhynchus* to experience exposure to large ostracod densities without substantial changes in resource availability. As a result, it is possible that there is a coexistence between *Ae. camptorhynchus* and ostracods in these habitats and consequentially partitioning of resources driven by differences in species' niches (Amarasekare 2003) and that the changes in adult size observed in these trials were driven by intraspecific competition although further investigation is necessary.

While it is important to realise the potential influence of controphic competition to mosquito development, survival and size, we must emphasise that laboratory experiments do not always reflect field populations. The outcome of competitive effects can change given different abiotic conditions (Tilman 1982, Costanzo et al. 2005, Juliano 2009). Such changes can be observed when habitats are drying out or with the addition of new invertebrates through rainfall or tide (Chase et al. 2003). For example, hatching of first instar larvae may differ to ostracods hatching from dormancy (Campos and Sy 2006, Khatchikian et al. 2010). Such a situation could result in a time window in which first instar mosquitoes have a competitive advantage both in size and nutrient acquisition. It might also be possible that different life stages of *Ae. camptorhynchus* larvae are more sensitive to environmental changes or intra-specific competition (Silberbush et al. 2014). Therefore, replicating these experiments in natural conditions in the field, with the addition of a fully crossed design (where all levels of resource limitation are also tested with ostracod densities against *Ae. camptorhynchus* development,

survival and size) would benefit our understanding on the relationship between *Ae. camptorhynchus* inter- and intra-specific interactions and provide further insight on the complexity of competitive systems.

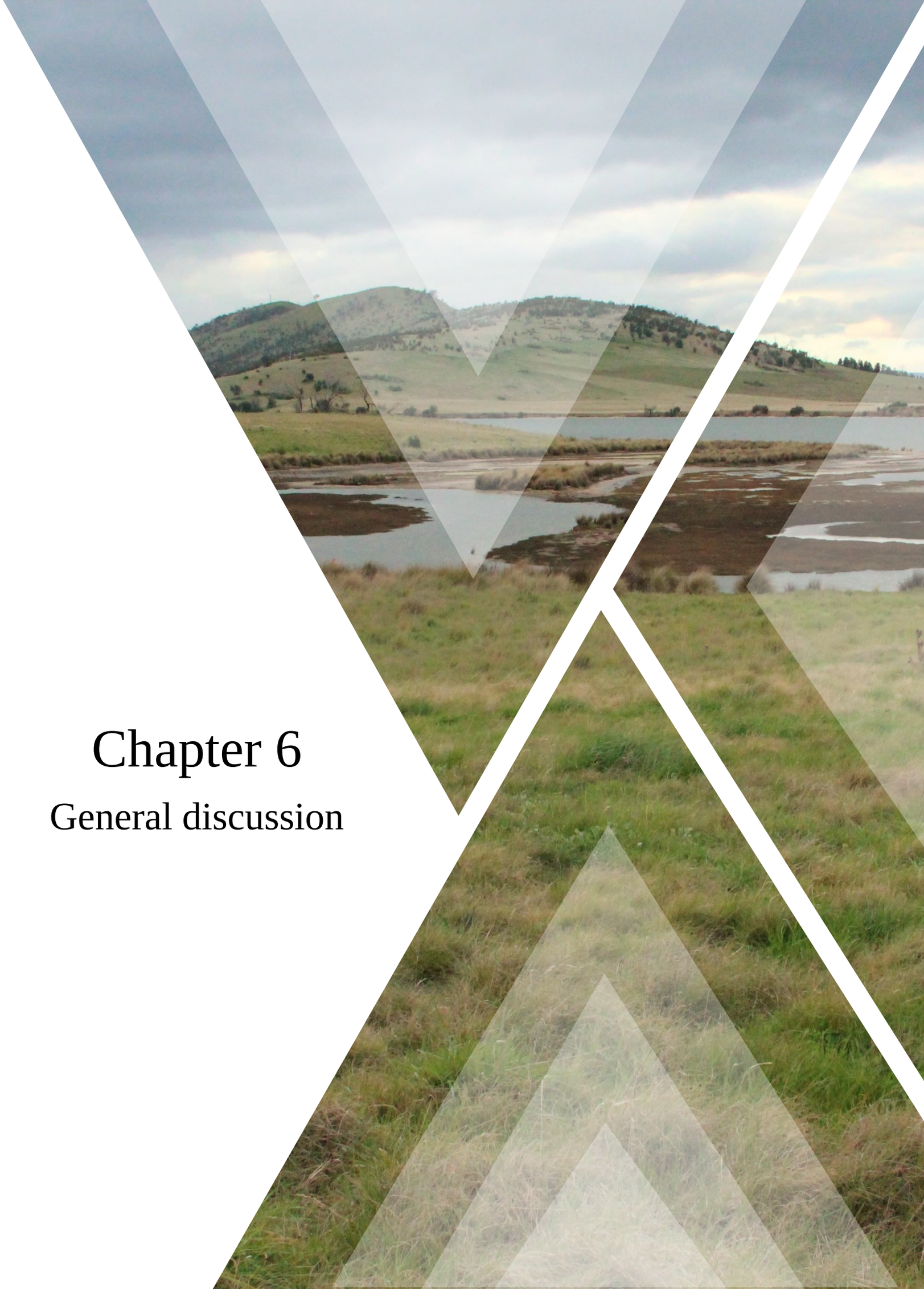
A limitation of our study is that we did not measure adult survival. Epidemiological models of mosquito-borne disease transmission incorporate adult longevity which can have significant effects on disease transmission (Reiskind and Lounibos 2009). However, environmental effects on larval development and survival have been demonstrated to be both condition- and species-specific, with strong associations between adult longevity, developmental times and body size demonstrated for some species (Mogi et al. 1996, Reiskind and Lounibos 2009). While resource limitation had a greater impact on *Ae. camptorhynchus* survival and development it is possible that densities of non-culidic competitors (and resource limitation) may result in reduced adult longevity especially considering both treatments had an effect on adult size.

We demonstrate that intra-specific resource limitation and controphic competition have a direct impact on adult sizes, however, changes to *Ae. camptorhynchus* life history in competitive environments is context dependent. We conclude that controphic competition, although quite complex, may have a role in influencing vector-borne disease and implications to human health.

Supplementary Information – Chapter 5

III. Mean and maximum developmental time and survival for *Ae. camptorhynchus* larvae by treatment for both resource limitation and competition experiments.

Treatment	Development		Survival	
Competition	Mean	Max	Mean	Max
0	29.2	37	71.3	100
150	24.1	33	60.8	92
300	24.6	38	61.2	80
600	29.8	39	72.6	84.2
Resource Limitation	Mean	Max	Mean	Max
0.6	19.3	21	96.1	100
0.4	25.8	37	86.6	100
0.2	36.2	37	77.4	91.7
0.1	57.3	70	61.5	68.4



Chapter 6

General discussion

Chapter 6 – General discussion

6.0 Thesis overview

Saltmarshes are important habitats globally for vector mosquitoes that transmit human disease (Russell and Dwyer 2000, Gubler 2002). In Australia, Ross River virus (RRv) is the most important vector-borne disease accounting for more than 5000 cases a year (Russell 1998). There are two mosquitoes, *Aedes camptorhynchus* and *Aedes vigilax,* that are responsible for most of the transmission across coastal regions of the country, with *Ae. camptorhynchus* occupying southern temperate saltmarshes and *Ae. vigilax* in northern tropical saltmarshes (Kokkinn et al. 2009). Prior to this work, several studies have investigated factors driving mosquito abundance and disease transmission including climatic drivers (Kelly-Hope et al. 2004, Tong et al. 2005), mosquito oviposition preferences (Gislason and Russell 1997, Dale et al. 2002, Knight et al. 2012), and mosquito management (Webb and Russell 1999, Dale and Breitfuss 2009, Rochlin et al. 2012), but the ecology of mosquitoes within saltmarshes is poorly understood. Furthermore, much of the current research regarding vector mosquitoes within Australia is focused on *Ae. vigilax.* with few studies covering *Ae. camptorhynchus* in temperate saltmarshes.

In this thesis, I focused on the ecology of *Ae. camptorhynchus* in temperate southern saltmarshes of Tasmania. Following an initial high-level literature review exploring historical areas of research on Australian saltmarsh vector mosquitoes (Chapter 2; Rowbottom et al. *in review*), I conducted an ecological comparative study and identified increased diversity of

aquatic invertebrates resulted in fewer vector mosquitoes, although there was variation in environmental factors in predicting *Ae. camptorhynchus* (first instar and pupal numbers), both within and between saltmarshes, reflecting the complexity of saltmarsh habitats (Chapter 3; Rowbottom et al. 2020). I then performed an environmental assessment of oviposition preferences of *Ae. camptorhynchus*, finding that vegetation (samphire) was the preferred substrate for oviposition and other parameters commonly observed with mosquito oviposition, such as soil moisture and presence of aquatic invertebrates, were less important (Chapter 4; Rowbottom et al. 2017). Finally, I utilised empirical laboratory data to investigate competitive and resource influences on vector mosquito life history. I discovered resources to be the primary limiting factor to mosquito life history, with inter-specific competition resulting in smaller adults (Chapter 5; Rowbottom et al. 2015). In summary, this thesis:

i. highlights the importance of saltmarsh ecology to vector mosquito abundance.
ii. identifies ecological complexity of spatially similar saltmarsh habitats and reveals aquatic determinants important to vector mosquito abundance in temperate saltmarsh habitats.
iii. demonstrates the importance of local saltmarsh hydrological knowledge on vector distribution.
iv. reveals dynamics of competitive interactions with vector mosquitoes

6.1 Summary of key findings

Urbanisation of coastal regions has increased the exposure of humans to saltmarshes and mosquito vectors. Not only has this resulted in greater mosquito transmitted disease but the health of saltmarsh habitats has been increasingly compromised (Adam 2002, Jardine et al. 2014). Healthy ecosystems provide significant value to humans through means of regulating the pests and disease (Weinstein 1997). However, from my literature review, I identified that saltmarsh ecosystems throughout Australia are largely understudied. I emphasise the applications gained from improving this fundamental knowledge of saltmarsh ecosystems; including better approaches to saltmarsh management, more efficient mosquito surveillance and improved understanding of other potential pathways for mosquito vector control.

Changes to the approach of saltmarsh management and vector control are emerging with focus on restoring saltmarsh habitats (Laegdsgaard 2006, Boon et al. 2015). Ecological information in these habitats is imperative to ensure restoration efforts are beneficial to ecosystem diversity and human health (Hanford et al. 2020). Saltmarshes are dynamic environments with habitat complexity between saltmarsh systems, leading to the inability of climatic predictive models to be applied universally between regions (Tong and Hu 2002, Yu et al. 2014, Koolhof et al. 2019), and discrepancies in mosquito surveillance and forecasting models (Cutcher et al. 2017). In my research, I have increased our understanding of the spatial and temporal ecological differences between temperate saltmarshes and how environmental conditions can impact immature *Ae. camptorhynchus*. Furthermore, my work demonstrates how improving site-specific knowledge of saltmarsh ecology can reveal how ecosystem health and aquatic invertebrate diversity can limit abundance of vector mosquitoes.

Previous studies have provided essential knowledge to *Ae. vigilax* distribution within saltmarshes (Evin and Talley 2000, Woodruff et al. 2003, Dale et al. 2008, Jacups et al. 2009,

Wisz et al. 2013) however, there has been little research understanding *Ae. camptorhynchus* distribution within temperate saltmarshes. There are many spatial complexities within saltmarsh systems which are attributed to the specific relationship the saltmarsh habitat has with hydrological events, in-situ climatic conditions, aquatic invertebrates and surrounding environmental factors (Saintilan 2009). The work presented in my thesis highlights how the key components driving vector abundance are context driven. While saltmarsh vegetation is an important component for *Ae. camptorhynchus* distribution, other known environmental indicators to mosquito distribution, particularly presence of aquatic competitors, were less important. Research within a temperate saltmarsh, known for high mosquito vector numbers, proposed potential implications surrounding aquatic competitors, ostracods, and *Ae. camptorhynchus* distribution and abundance (Carver et al. 2011). Although, I reveal that not only is the distribution of ostracods expansive within this saltmarsh, but their influence on *Ae. camptorhynchus* abundance is primarily through limiting resources and, in larger numbers, limiting development time.

6.2 Management implications

Saltmarshes are valuable ecosystems which are globally under threat of habitat loss and declining ecosystem function (Corvalán et al. 2005, Knight et al. 2017, Himes-Cornell et al. 2018). Many global issues with increased zoonotic diseases are associated with declining ecosystem health (Corvalán et al. 2005). Furthermore, with the continual disruptions of these ecosystems, and proximity of residential developments to these habitats, the incidence of vector-borne disease will continue to rise (Norris 2004). Within Australia, the epidemiological, and environmental factor driving mosquito-borne disease is extensive, both with habitat changes and vector differences (Claflin and Webb 2015) and there have been recommendations

for more local scale investigations to improve mosquito vector management (Russell and Kay 2004). My work provides valuable insight into the local dynamics driving mosquito abundance and distribution by demonstrating how ecological differences between saltmarshes with various degrees of external influences can impact vector mosquitoes. In particular, habitats with decreased aquatic ecological diversity presented higher risk of greater numbers of vector mosquitoes. Therefore, future management of saltmarsh habitats for vector control may consider understanding the aquatic macroinvertebrate ecosystem communities by implementing aquatic diversity evaluations that are comparable to undisturbed habitats. Furthermore, development of these ecosystems will need to implement ways to avoid ecosystem function disruptions as this will increase the risk to human health.

Across Australia, there is a considerable amount of saltmarsh variability and complexity, each with unique associations with hydrology, connectivity, and invertebrate aquatic diversity (Saintilan 2009). Many local studies of saltmarsh habitats and the mosquito vectors within these environments contribute to current and future vector management. For example, improved knowledge of the most productive habitat and environmental conditions for vectors to emerge from, and thereby target, benefit control efforts. Often, mosquito distribution is associated with environmental factors, such as vegetation, moisture and presence of predators or competitors (Dale et al. 1986, Gislason and Russell 1997, Dale et al. 2008, Jacups et al. 2009). I reveal that, although vegetation may remain a driving factor in mosquito oviposition, the suitability of these habitats may differ throughout the saltmarsh according to specific hydrological conditions. While aquatic invertebrates may influence oviposition, mosquito distribution and abundance are highly variable within and between saltmarshes, and the nature of interactions not always straightforward. With the spatial variability displayed by *Ae. camptorhynchus* in temperate saltmarshes, there are several future management strategies that can be implemented. Knowledge on specific tidal connectivity throughout the saltmarsh would

provide a better understanding of primary mosquito habitat for targeted control. Moreover, rehabilitation of tidal connectivity would contribute towards passive vector control by increased water movement through the saltmarsh dispersing mosquito vectors exposing them to increased predation or flushing them from the saltmarsh.

6.3 Broader applications

Ecological applications revealed in this thesis are not limited to *Ae. camptorhynchus*, but can be applied to other vector mosquitoes important to human transmitted disease, as well as other aquatic systems where vector mosquitoes reside. Mosquitoes of global importance occupy a range of aquatic habitats from freshwater container breeding mosquitoes to either inland or coastal saline mosquitoes (Dale and Knight 2008). The benefits gained from monitoring and evaluating ecosystems include insights in understanding cycles of disease and potential mechanisms underlying mosquito outbreaks in other systems. In addition, this research has demonstrated that revealing the underlying ecological factors driving mosquito abundance between saltmarshes has future applications in both ecological restoration and rehabilitation measures and targeted mosquito control programs for the benefit of ecosystem biodiversity and human health.

Efforts to improve saltmarsh management and restoration would also benefit from understanding local saltmarsh aquatic diversity. Currently, there is very little known about the aquatic diversity in saltmarsh habitats and improving the taxonomic diversity through repairing ecosystem health of these regions, will have cascading effects on mosquito vector abundance. Furthermore, understanding the invertebrate dynamics within saltmarshes can be used as biodiversity indicators of ecosystem health which can be applied to other local, or regional saltmarshes. As climatic conditions continue to change, the dynamics of saltmarsh ecosystems

and vector mosquitoes will also change. Therefore, continued development of mosquito vector research is necessary to detect and understand how these changes will impact the future of vector-borne disease.

6.4 Future recommendations

The research presented in this thesis provide relevant information for the advancement of saltmarsh management and vector mosquito control. For many regions across Australia there is a lack of knowledge surrounding local environmental factors driving the ecology of saltmarsh system and the mosquitoes within them. More research is required on local hydrological cycles, in-situ environmental factors and aquatic invertebrate assemblages which would allow researchers to better direct management approaches for vector mosquito control.

In a local context, this thesis has identified distinct differences in vector mosquito abundance between local saltmarshes and the underlying ecological factors contributing to these differences. Future research should be directed to improve our knowledge of vector mosquito distribution across the state to identify prominent regions for potential disease outbreaks. Also, in these areas identified, preliminary research is required to understand the aquatic ecosystem which will provide a baseline in our understanding of the ecosystem health of these areas which can be monitored and managed to minimise the potential outbreak of disease.

6.5 Conclusion

The research presented in this thesis contributes substantially to our understanding of saltmarsh ecology and how diverse and healthy ecosystems impact vector mosquitoes in temperate saltmarshes. Few studies have explored the diversity between saltmarsh aquatic ecosystems or

incorporated this knowledge in regular surveillance methods to better understand vector dynamics. To improve our ability to predict, manage and control mosquito outbreaks and transmission of human disease, additional research is needed to understand ecological factors that underpin mosquito abundance. In addition, this thesis has exposed how the spatial difference in mosquito distribution is dependent on specific saltmarsh factors which drive fine-scale mosquito distribution and that aquatic interactions are context dependent. With the complexity of saltmarsh systems, climatic conditions and diversity of mosquito vectors, understanding the local dynamics of saltmarsh ecology and the changes in these habitats that drive mosquito abundance will allow researchers to reduce mosquito outbreaks and prevent human disease.

Literature cited

Literature cited

Aaskov J, Fokine A, Liu WJ 2012. Ross River virus evolution: Implications for vaccine development. *Future Virology* 7:173-178

Aaskov JG, Mataika JU, Lawrence GW, Rabukawaqa V, Tucker M, Miles JA, Dalglish DA 1981. An epidemic of Ross River virus infection in Fiji, 1979. *American Journal of Tropical Medicine and Hygiene* 30:1053-1059

Aaskov JG, Ross PV, Harper JJ, Donaldson MD 1985. Isolation of Ross River virus from epidemic polyarthritis patients in Australia. *Australian Journal of Experimental Biology and Medical Science* 63:587-597

Adam P 1990. Saltmarsh Ecology (Cambridge studies in ecology), Cambridge: Cambridge University Press, pp 461

Adam P 2002. Saltmarshes in a time of change. *Environmental Conservation* 29:39-61

Alexandridis N, Bacher C, Desroy N, Jean F 2017. Building functional groups of marine benthic macroinvertebrates on the basis of general community assembly mechanisms. *Journal of Sea Research* 121:59-70

Alto BW, Lounibos LP, Higgs S, Juliano SA 2005. Larval competition differentially affects arbovirus infection in *Aedes* mosquitoes. *Ecology* 86:3279-3288

Amarasekare P 2003. Competitive coexistence in spatially structured environments: A synthesis. *Ecology Letters* 6:1109-1122

Andreadis TG 1990. Observations on installment egg hatching in the brown salt-marsh mosquito, *Aedes-cantator*. *Journal of the American Mosquito Control Association* 6:727-729

Bader CA, Williams CR 2011. Eggs of the Australian saltmarsh mosquito, *Aedes camptorhynchus*, survive for long periods and hatch in instalments: Implications for biosecurity in New Zealand. *Medical and Veterinary Entomology* 25:70-76

Ballard JWO, Marshall ID 1986. An investigation of the potential of *Aedes camptorhynchus* (Thom) as a vector of Ross River virus. *Australian Journal of Experiemntal Biology and Medical Science* 64:197-200

Banerjee S, Aditya G, Saha N, Saha GK 2010. An assessment of macroinvertebrate assemblages in mosquito larval habitats - Space and diversity relationship. *Environmental Monitoring and Assessment* 168:597-611

Bara J, Rapti Z, Cáceres CE, Muturi EJ 2015. Effect of larval competition on extrinsic incubation period and vectorial capacity of *Aedes albopictus* for Dengue virus. *PLoS one* 10:e0126703

Barrera R 1996. Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. *Ecological Entomology* 21:117-127

Barton PS, Aberton JG 2005. Larval development and autogeny in *Ochlerotatus camptorhynchus* (Thomson) (Diptera: Culicidae) from southern Victoria. *Proceedings of the Linnean Society of New South Wales* 126:261-267

Barton PS, Aberton JG, Kay BH 2004. Spatial and temporal definition of *Ochlerotatus camptorhynchus* (Thomson) (Diptera: Culicidae) in the Gippsland Lakes system of eastern Victoria. *Australian Journal of Entomology* 43:16-22

Beketov MA, Liess M 2007. Predation risk perception and food scarcity induce alterations of life-cycle traits of the mosquito *Culex pipens*. *Ecological Entomology* 32:405-410

Bentley MD, Day JF 1989. Chemical ecology and behavioral-aspects of mosquito oviposition. *Annual Review of Entomology* 34:401-421

Bevins SN 2007. Timing of resource input and larval competition between invasive and native container-inhabiting mosquitoes (Diptera: Culicidae). *Journal of Vector Ecology* 32:252-262

Bi P, Parton KA, Kevin A 2003. Climate variations and the transmission of Ross River virus infection in coastal and inland region of Queensland: An analysis from Townsville and Toowoomba. *Environmental Health* 3:73-79

Blackmore MS, Lord CC 2000. The relationship between size and fecundity in *Aedes albopictus*. *Journal of Vector Ecology* 25:212-217

Blaustein L, Chase JM 2007. Interactions between mosquito larvae and species that share the same trophic level. *Annual Review of Entomology* 52:489-507

Bond EM and Chase JM 2002. Biodiversity and ecosystem functioning at local and regional spatial scales. *Ecology Letters* 5:467-470

Boon PI, Allen T, Carr G, Frood D, Harty C, Mcmahon A, Mathews S, Rosengren N, Sinclair S, White M, Yugovic J 2015. Coastal wetlands of Victoria, south-eastern Australia: Providing the inventory and condition information needed for their effective management and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 25:454-479

Boorman LA 1999. Salt marshes – Present functioning and future change. *Mangroves and Salt Marshes* 3:227-241

Boulton AJ, Lloyd LN 1992. Flooding frequency and invertebrate emergence from dry floodplain sediments of the River Murray, Australia. *Regulated Rivers: Research & Management* 7:137-151

Brady VJ, Cardinale BJ, Gathman JP, Burton TM 2002. Does facilitation of faunal recruitment benefit ecosystem restoration? an experimental study of invertebrate assemblages in wetland mesocosms. *Restoration Ecology* 10:617-626

Breiman L 2001. Random Forests. *Machine Learning* 45:5-32

Brown MD, Carter J, Watson TM, Thomas P, Santaguliana G, Purdie DM, Kay BH 2001. Evaluation of liquid *Bacillus thuringiensis var. israelensis* products for control of Australian *Aedes* arbovirus vectors. *Journal of the American Mosquito Control Association* 17:8-12

Brown MD, Thomas D, Mason P, Greenwood JG, Kay BH 1999. Laboratory and field evaluation of the efficacy of four insecticides for *Aedes vigilax* (Diptera: Culicidae) and toxicity to the nontarget shrimp *Leander tenuicornis* (Decapoda: Palaemonidae). *Journal of Economic Entomology* 92:1045-1051

Brown MD, Thomas D, Watson K, Greenwood JG, Kay BH 1996. Acute toxicity of selected pesticides to the estuarine shrimp *Leander tenuicornis* (Decapoda: Palaemonidae). *Journal of the American Mosquito Control Association* 12:721-724

Brown MD, Thomas D, Watson K, Kay BH 1998. Laboratory and field evaluation of efficacy of VectoBac 12AS against *Culex sitiens* (Diptera: Culicidae) larvae. *Journal of the American Mosquito Control Association* 14:183-5

Cadotte MW, Carscadden K, Mirotchnick N 2011. Beyond species: Functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology* 48:1079-1087

Campos RE, Sy VE 2006. Variation in the hatching response of *Ochlerotatus albifasciatus* egg batches (Diptera: Culicidae) in temperate Argentina. *Memorias Do Instituto Oswaldo Cruz* 101:47-53

Cardo MV, Vezzani D, Carbajo AE 2012. Oviposition strategies of temporary pool mosquitoes in relation to weather, tidal regime and land use in a temperate wetland. *Bulletin of Entomological Research* 102:651-662

Carver S, Goater S, Allen GR, Parr R, Fearnley E, Weinstein P 2011. Relationships of the Ross River virus (Togoviridae: *Alphavirus*) vector, *Aedes camptorhynchus* (Thomson) (Diptera: Culicidae), to biotic and abiotic factors in saltmarshes of south-eastern Tasmania, Australia: a preliminary study. *Australian Journal of Entomology* 50:344-355

Carver S, Kilpatrick AM, Kuenzi A, Douglass R, Ostfeld RS, Weinstein P 2010a. Environmental monitoring to enhance comprehension and control of infectious diseases. *Journal of Environmental Monitoring* 12:2048-2055

Carver S, Spafford H, Storey A, Weinstein P 2009. Colonization of ephemeral water bodies in the wheatbelt of Western Australia by assemblages of mosquitoes (Diptera: Culicidae): Role of environmental factors, habitat and disturbance. *Environmental Entomology* 38:1585-1594

Carver S, Spafford H, Storey A, Weinstein P 2010b. The roles of predators, competitors, and secondary salinization in structuring mosquito (Diptera: Culicidae) assemblages in ephemeral water bodies of the Wheatbelt of Western Australia. *Environmental Entomology* 39:798-810

Chapman HF, Hughes JM, Jennings C, Kay BH, Ritchie SA 1999. Population structure and dispersal of the saltmarsh mosquito *Aedes vigilax* in Queensland, Australia. *Medical and Veterinary Entomology* 13:423-430

Chase JM, Knight TM 2003. Drought-induced mosquito outbreaks in wetlands. *Ecology Letters* 6:1017-1024

Chaves LF, Keogh CL, Nguyen AM, Decker GM, Vazquez-Prokopec GM, Kitron UD 2011. Combined sewage overflow accelerates immature development and increases body size in the urban mosquito *Culex quinquefasciatus*. *Journal of Applied Entomology* 135:611-620

Claflin SB, Webb CE 2015. Ross River virus: Many vectors and unusual hosts make for an unpredictable pathogen. *PLoS pathogens* 11:e1005070

Claflin SB, Webb CE 2017. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. *Wetlands Ecology and Management* 25:331-344

Clark TM, Flis BJ, Remold SK 2004. Differences in the effects of salinity on larval growth and developmental programs of a freshwater and a euryhaline mosquito species (Insecta: Diptera, Culicidae). *Journal of Experimental Biology* 207:2289-2295

Clements AN 1999. The biology of mosquitoes, volume 1: Development, nutrition and reproduction. Wallingford, United Kingdom: Chapman and Hall, pp 509

Cook A, Jardine A, Weinstein P 2004. Using human disease outbreaks as a guide to multilevel ecosystem interventions. *Environmental Health Perspectives* 112:1143-1146

Corvalán C, Hales S, McMichael AJ 2005. Ecosystems and human well-being: Health synthesis, millennium ecosystem assessment. Geneva, Switzerland: World Health Organization, pp 53

Costanzo KS, Kesavaraju B, Juliano SA 2005. Condition-specific competition in container mosquitoes: The role of noncompeting life-history stages. *Ecology* 86:3289-3295

Cummins KW, Klung MJ 1979. Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* 10:147-172

Cutcher Z, Williamson E, Lynch SE, Rowe S, Clothier HJ, Firestone SM 2017. Predictive modelling of Ross River virus notifications in southeastern Australia. *Epidemiology and Infection* 145:440-450

Cutler DR, Edwards Jr. TC, Beard KH, Cutler A, Hess KT, Gibson J, Lawler JJ 2007. Random Forests for classification in ecology. *Ecology* 88:2783-2792

Dale P, Breitfuss M 2009. Ecology and management of mosquitoes. In: Saintilan, N, *Australian Saltmarsh Ecology,* Australia: CSIRO Publishing, pp 167-178

Dale P, Knight J, Griffin L 2014. Comparing *Aedes vigilax* eggshell densities in saltmarsh and mangrove systems with implications for management. *Insects* 5:984-990

Dale PE, Ritchie SA, Chapman H, Brown MD 1999. Eggshell sampling: Quantitative or qualitative data? *Journal of the American Mosquito Control Association* 15:74-6

Dale PER 2008. Assessing impacts of habitat modification on a subtropical salt marsh: 20 years of monitoring. *Wetlands Ecology and Management* 16:77-87

Dale PER, Chapman H, Brown MD, Ritchie SA, Knight J, Kay BH 2002. Does habitat modification affect oviposition by the saltmarsh mosquito *Ochlerotatus vigilax* (Skuse) (Diptra: Culicidae)? *Australian Journal of Entomology* 41:49-54

Dale PER, Dale PT, Hulsman K, Kay BH 1993. Runnelling to control salt-marsh mosquitoes - long term efficacy and environmental impacts. *Journal of the American Mosquito Control Association* 9:174-181

Dale PER, Hulsman K, Harrison D, Congdon B 1986. Distribution of the immature stages of *Aedes-vigilax* on a coastal salt-marsh in southeast Queensland. *Australian Journal of Ecology* 11:269-278

Dale PER, Knight J, Kay BH, Chapman H, Ritchie SA, Brown MD 2008. Habitat characteristics and eggshell distribution of the salt marsh mosquito, *Aedes vigilax*, in marshes in subtropical eastern Australia. *Journal of Insect Science* 8:1-8

Dale PER, Knight JM 2006. Managing salt marshes for mosquito control: Impacts of runnelling, open marsh water management and grid-ditching in sub-tropical Australia. *Wetlands Ecology and Management* 14:211-220

Dale PER, Knight JM 2008. Wetlands and mosquitoes: A review. *Wetlands Ecology and Management* 16:255-276

Dale PER, Knight JM 2012. Managing mosquitoes without destroying wetlands: An eastern Australian approach. *Wetlands Ecology and Management* 20:233-242

Dale PER, Ritchie SA, Territo BM, Morris CD, Muhar A, Kay BH 1998. An overview of remote sensing and GIS for surveillance of mosquito vector habitats and risk assessment. *Journal of Vector Ecology* 23:54-61

Daugherty MP, Juliano SA 2003. Leaf scraping beetle feces are a food resource for tree hole mosquito larvae. *American Midland Naturalist* 150:181-184

de Little SC, Bowman D, Whelan PI, Brook BW, Bradshaw CJA 2009. Quantifying the drivers of larval density patterns in two tropical mosquito species to maximize control efficiency. *Environmental Entomology* 38:1013-1021

Dhileepan K, Peters C, Porter A 1997. Prevalence of *Aedes camptorhynchus* (Thomson) (Diptera: Culicidae) and other mosquitoes in the eastern coast of Victoria. *Australian Journal of Entomology* 36:183-190

Doherty RL, Carley JG, Best JC 1972. Isolation of Ross River virus from man. *Medical Journal of Australia* 1:1083-1084

Duquesne S, Kroeger I, Kutyniok M, Liess M 2011. The potential of cladocerans as controphic competitors of the mosquito *Culex pipiens*. *Journal of Medical Entomology* 48:554-560

Eder M, Cortes F, Filha NTD, de Franca GVA, Degroote S, Braga C, Ridde V, Martelli CMT 2018. Scoping review on vector-borne diseases in urban areas: Transmission dynamics, vectorial capacity and co-infection. *Infectious Diseases of Poverty* 7:90

Eitam A, Blaustein L 2004. Oviposition habitat selection by mosquitoes in response to predator (*Notonecta maculata*) density. *Physiological Entomology* 29:188-191

Eitam A, Blaustein L, Van Damme K, Dumont HJ, Martens K 2004. Crustacean species richness in temporary pools: Relationships with habitat traits. *Hydrobiologia* 525:125-130

ESRI 2014. ArcGIS Desktop. *Environmental Systems Research Institute.* 10.2 ed. Redlands, California.

Evin LA, Talley TS 2000. Influences of vegetation and abiotic environmental factors on salt marsh invertebrates. In: Weinstein, MP & Kreeger, DA, *Concepts and Controversies in Tidal Marsh Ecology* Dordrecht, Netherlands: Springer, pp 661-707

Farjana T, Tuno N, Higa Y 2012. Effects of temperature and diet on development and interspecies competition in *Aedes aegypti* and *Aedes albopictus*. *Medical and Veterinary Entomology* 26:210-217

Ford H, Garbutt A, Jones L, Jones DL 2013. Grazing management in saltmarsh ecosystems drives invertebrate diversity, abundance and functional group structure. *Insect Conservation and Diversity* 6:189-200

Gedan KB, Silliman BR, Bertness MD 2009. Centuries of human-driven change in salt marsh ecosystems. *Annual Review of Marine Science* 1:117-141

Gelman A, Hill J 2006. Data analysis using regression and multilevel/hierarchial models, United Kingdom: Cambridge University Press, pp 654

Gilles JRL, Lees RS, Soliban SM, Benedict MQ 2011. Density-dependent effects in experimental larval populations of *Anopheles arabiensis* (Diptera: Culicidae) can be negative, neutral, or overcompensatory depending on density and diet levels. *Journal of Medical Entomology* 48:296-304

Gillett JD 1971. Mosquitos, The world naturalist, London: Weidenfeld and Nicolson, pp 174

Gislason GM, Russell RC 1997. Oviposition sites of the saltmarsh mosquito, *Aedes vigilax* (Skuse) (Diptera: Culicidae), at Homebush Bay, Sydney, NSW - a preliminary investigation. *Australian Journal of Entomology* 36:97-100

Gonzalez PV, Audino PAG, Masuh HM 2016. Oviposition behavior in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in response to the presence of heterospecific and conspecific larvae. *Journal of Medical Entomology* 53:268-272

Gratz NG 1999. Emerging and resurging vector-borne diseases. *Annual Review of Entomology* 44:51-75

Griffin LF, Knight JM, Dale PER 2010. Identifying mosquito habitat microtopography in an Australian mangrove forest using LiDAR derived elevation data. *Wetlands* 30:929-937

Gubler DJ 2001. Human arbovirus infections worldwide. *Annals of the New York Academy of Science* 951:13-24

Gubler DJ 2002. The global emergence/resurgence of arboviral diseases as public health problems. *Archives of Medical Research* 33:330-342

Hadfield JD 2010. MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software* 33:22

Hanford JK, Webb CE, Hochuli DF 2020. Management of urban wetlands for conservation can reduce aquatic biodiversity and increase mosquito risk. *Journal of Applied Ecology;* DOI: 10.1111/1365-2664.13576

Harley D, Ritchie S, Phillips D, van den Hurk A 2000. Mosquito isolates of Ross River virus from Cairns, Queensland, Australia. *American Journal of Tropical Medicine & Hygiene* 62:561-565

Harley D, Sleigh A, Ritchie S 2001. Ross River virus transmission, infection, and disease: A cross-disciplinary review. *Clinical Microbiology Reviews* 14:909-932

Haynert K, Kiggen M, Klarner B, Maraun M, Scheu S 2017. The structure of salt marsh soil mesofauna food webs – The prevalence of disturbance. *PLoS one* 12:e0189645

Himes-Cornell A, Pendleton L, Atiyah P 2018. Valuing ecosystem services from blue forests: A systematic review of the valuation of salt marshes, sea grass beds and mangrove forests. *Ecosystem Services* 30:36-48

Hugo LE, Kay BH, Ryan PA 2003. Autogeny in *Ochlerotatus vigilax* (Diptera: Culicidae) from southeast Queensland, Australia. *Journal of Medical Entomology* 40:897-902

Hulsman K, Dale PE, Kay BH 1989. The runnelling method of habitat modification: An environment-focused tool for salt marsh mosquito management. *Journal of the American Mosquito Control Association* 5:226-34

Jacups S, Warchot A, Whelan P 2012. Anthropogenic ecological change and impacts on mosquito breeding and control strategies in salt-marshes, Northern Territory, Australia. *Ecohealth* 9:183-194

Jacups SP, Carter J, Kurucz N, McDonnell J, Whelan PI 2015. Determining meteorological drivers of salt marsh mosquito peaks in tropical northern Australia. *Journal of Vector Ecology* 40:277-281

Jacups SP, Kurucz N, Whelan PI, Carter JM 2009. A comparison of *Aedes vigilax* larval population densities and associated vegetation categories in a coastal wetland, Northern Territory, Australia. *Journal of Vector Ecology* 34:311-316

Jacups SP, Whelan PI, Currie BJ 2008a. Ross River virus and Barmah Forest virus infections: A review of history, ecology, and predictive models, with implications for tropical northern Australia. *Vector-Borne and Zoonotic Diseases* 8:283-297

Jacups SP, Whelan PI, Markey PG, Cleland SJ, Williamson GJ, Currie BJ 2008b. Predictive indicators for Ross River virus infection in the Darwin area of tropical northern Australia, using long-term mosquito trapping data. *Tropical Medicine & International Health* 13:943-952

Janes R, Jardine A, Neville PJ, Nicholson J, Lindsay MDA 2018. Mosquito (Diptera: Culicidae) communities of metropolitan Perth, Western Australia, and implications for managing pest and public health risks. *Austral Entomology* 57:324-332

Jardine A, Neville PJ, Dent C, Webster C, Lindsay MDA 2014. Ross River virus risk associated with dispersal of *Aedes* (Ochlerotatus) *camptorhynchus* (Thomson) from breeding habitat into surrounding residential areas: Muddy Lakes, Western Australia. *The American Journal of Tropical Medicine and Hygiene* 91:101-108

Jeffery JAL, Kay BH, Ryan PA 2006. Role of *Verrallina funerea* (Diptera: Culicidae) in transmission of Barmah forest virus and Ross River virus in coastal areas of eastern Australia. *Journal of Medical Entomology* 43:1239-1247

Jennings CD, Kay BH 1999. Dissemination barriers to Ross River virus in *Aedes vigilax* and the effects of larval nutrition on their expression. *Medical and Veterinary Entomology* 13:431-438

Johnston E, Weinstein P, Slaney D, Flies AS, Fricker S, Williams C 2014. Mosquito communities with trap height and urban-rural gradient in Adelaide, South Australia: Implications for disease vector surveillance. *Journal of Vector Ecology* 39:48-55

Juliano SA 1998. Species introduction and replacement among mosquitoes: Interspecific resource competition or apparent competition? *Ecology* 79:255-268

Juliano SA 2007. Population dynamics. *Journal of the American Mosquito Control Association* 23 (Supp. 2); 265-275

Juliano SA 2009. Species interactions among larval mosquitoes: Context dependence across habitat gradients. *Annual Review of Entomology* 54:37-56

Kay BH, Ferguson KJ, Morgan RNC 1973. Control of salt-marsh mosquitos with abate insecticide at Coombabah-Lakes, Queensland, Australia. *Mosquito News* 33:529-535

Kay BH, Jennings CD 2002. Enhancement or modulation of the vector competence of *Ochlerotatus vigilax* (Diptera: Culicidae) for Ross River virus by temperture. *Journal of Medical Entomology* 39:99-105

Kay BH, Jorgensen WK 1986. Eggs of *Aedes vgilax* (Skuse) and their distribution on plants and soil in south east Queensland saltmarsh. *Australian Journal of Entomology* 25:267-272

Kay BH, Russell RC 2013. Mosquito eradication: The story of killing campto, Victoria: CSIRO Publishing, pp 279

Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, Myers SS, Bogich T, Ostfeld RS 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 468:647-652

Kelly-Hope LA, Purdie DM, Kay BH 2004. Differences in climatic factors between Ross River virus disease outbreak and nonoutbreak years. *Journal of Medical Entomology* 41:1116-1122

Khatchikian CE, Dennehy JJ, Vitek CJ, Livdahl TP 2010. Environmental effects on bet hedging in *Aedes* mosquito egg hatch. *Evolutionary Ecology* 24:1159-1169

Knight, K.L., and T.E. Baker. 1962. The role of the substrate moisture content in the selection of oviposition sites by *Aedes taeniorhynchus* (Wied.) and *Ae. sollicitans* (Walk.). *Mosquito News* 22:247-254

Knight J, Dale P, Dwyer P, Marx S 2017. A conceptual approach to integrate management of ecosystem service and disservice in coastal wetlands. *Aims Environmental Science* 4:431-442

Knight J, Griffin L, Dale P, Phinn S 2012. Oviposition and larval habitat preferences of the saltwater mosquito, *Aedes vigilax*, in a subtropical mangrove forest in Queensland, Australia. *Journal of Insect Science* 12:6

Knight JM 2011. A model of mosquito–mangrove basin ecosystems with implications for management. *Ecosystems* 14:1382-1395

Knight TM, Chase JM, Goss CW, Knight JJ 2004. Effects of interspecific competition, predation, and their interaction on survival and development time of immature *Anopheles quadrimaculatus*. *Journal of Vector Ecology* 29:277-284

Kokkinn MJ, Duval DJ, Williams CR 2009. Modelling the ecology of the coastal mosquitoes *Aedes vigilax* and *Aedes camptorhynchus* at Port Pirie, South Australia. *Medical and Veterinary Entomology* 23:85-91

Koolhof IS, Bettiol S, Carver S 2017. Fine-temporal forecasting of outbreak probability and severity: Ross River virus in Western Australia. *Epidemiology and Infection* 145:2949-2960

Koolhof IS, Gibney KB, Bettiol S, Charleston M, Wiethoelter A, Arnold A-L, Campbell PT, Neville PJ, Aung P, Shiga T, Carver S, Firestone SM 2020. The forecasting of dynamical Ross River virus outbreaks: Victoria, Australia. *Epidemics.* DOI: org/10.1016/j.epidem.2019.100377

Kurucz N, Whelan PI, Carter JM, Jacups SP 2009. A geospatial evaluation of *Aedes vigilax* larval control efforts across a coastal wetland, Northern Territory, Australia. *Journal of Vector Ecology* 34:317-323

Laegdsgaard P 2006. Ecology, disturbance and restoration of coastal saltmarsh in Australia: A review. *Wetlands Ecology and Management* 14:379-399

Lee DJ 1949. Mosquitoes (Diptera, Culicidae) Recorded from Tasmania. *Records of the Queen Victoria Museum Launceston.* Launceston: Museum Committee Launceston City Council.

Leo KL, Gillies CL, Fitzsimons JA, Hale LZ, Beck MW 2019. Coastal habitat squeeze: A review of adaptation solutions for saltmarsh, mangrove and beach habitats. *Ocean & Coastal Management* 175:180-190

Liaw A, Wiener, M. 2002. Classification and regression by randomForest. *R News* 2:18-27

Liu X, Tharmarajah K, Taylor A 2017. Ross River virus disease clinical presentation, pathogenesis and current therapeutic strategies. *Microbes and Infection* 19:496-504

Lounibos LP 1994. Geographical and developmental components of adult size of neotropical *Anopheles (Nyssorhynchus). Ecological Entomology* 19:138-146

Lounibos LP 2002. Invasions by insect vectors of human disease. *Annual Review of Entomology* 47:233-266

Lyth A, Holbrook NJ 2015. Assessing an indirect health implication of a changing climate: Ross River virus in a temperate island state. *Climate Risk Management* 10:77-94

Lyth A, Holbrook NJ, Beggs PJ 2005. Climate, urbanisation and vulnerability to vector-borne disease in subtropical coastal Australia: Sustainable policy for a changing environment. *Global Environmental Change Part B: Environmental Hazards* 6:189-200

McCall BD, Pennings SC 2012. Geographic variation in salt marsh structure and function. *Oecologia* 170:777-787

McManus T 1994. Ross river virus: A review based on Tasmanian experience. *Australian Institute of Environmental Health.* Tasmania, p8

McManus T, Marshall ID 1986. The epidemology of Ross River Virus in Tasmania. *Arbovirus Research in Australia* 4:127-131

McMichael AJ, Lindgren E 2011. Climate change: Present and future risks to health, and necessary responses. *Journal of Internal Medicine* 270:401-413

Merritt RW, Dadd RH, Walker ED 1992. Feeding behaviour, natural food, and nutritional relationships of larval mosquitos. *Annual Review of Entomology* 37:349-376

Miller RJ, Wing J, Cope S, Davey RB, Kline DL 2005. Comparison of carbon dioxide- and octenol-baited encephalitis virus surveillance mosquito traps at the Shoal Water Bay

Training area, Queensland, Australia. *Journal of the American Mosquito Control Association* 21:497-500

Mitchell-Foster K, Ma BO, Warsame-Ali S, Logan C, Rau ME, Lowenberger C 2012. The influence of larval density, food stress, and parasitism on the bionomics of the dengue vector *Aedes aegypti* (Diptera: Culicidae): Implications for integrated vector management. *Journal of Vector Ecology* 37:221-229

Mogi M, Miyagi I, Abadi K, Syafruddin 1996. Inter- and intraspecific variation in resistance to desiccation by adult *Aedes* (Stegomyia) spp. (Diptera: Culicidae) from Indonesia. *Journal of Medical Entomology* 33:53-57

Mokany A, Shine R 2003a. Competition between tadpoles and mosquito larvae. *Oecologia* 135:615-620

Mokany A, Shine R 2003b. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. *Austral Ecology* 28:33-37

Molnar C, Bischl B, Casalicchio G 2018. iml: An R Package for interpretable machine learning. *The Journal of Open Source Software* 3:786

Morin PJ 1999. Community ecology, Massachusetts, USA: Blackwell Science, Inc., pp 406

Muturi EJ, Kim C-H, Alto BW, Berenbaum MR, Schuler MA 2011. Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. *Tropical Medicine & International Health* 16:955-964

Nasci RS, Mitchell CJ 1994. Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. *Journal of Medical Entomology* 31:123-126

Navarro-Silva MA, Marques FA, Duque JE 2009. Review of semiochemicals that mediate the oviposition of mosquitoes: A possible sustainable tool for the control and monitoring of Culicidae. *Revista Brasileira De Entomologia* 53:1-6

NNDSS 2019. National Notifiable Diseases Surveillance System database [Online]. Department of Health: Australian Government. Available: http://www9.health.gov.au/cda/source/Rpt_4_sel.cfm [accessed 12 January 2020]

Norris DE 2004. Mosquito-borne diseases as a consequence of land use change. *EcoHealth* 1:19-24

O'Sullivan L, Jardine A, Cook A, Weinstein P 2008. Deforestation, mosquitoes, and ancient Rome: Lessons for today. *BioScience* 58:756-760

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H 2019. Vegan: Community ecology package: Ordination, Diversity and Dissimilarities. 2.5-4 ed. R Package.

Ostfeld RS, Keesing F 2000. The function of biodiversity in the ecology of vector-borne zoonotic diseases. *Canadian Journal of Zoology* 78:2061-2078

Paaijmans KP, Blanford S, Chan BHK, Thomas MB 2012. Warmer temperatures reduce the vectorial capacity of malaria mosquitoes. *Biology Letters* 8:465-468

Packer MJ, Corbet PS 1989. Size variation and reproductive success of female *Aedes punctor* (Diptera: Culicidae). *Ecological Entomology* 14:297-309

Padmanabha H, Bolker B, Lord CC, Rubio C, Lounibos LP 2011. Food availability alters the effects of larval temperature on *Aedes aegypti* growth. *Journal of Medical Entomology* 48:974-984

Pickering C, Grignon J, Steven R, Guitart D, Byrne J 2015. Publishing not perishing: How research students transition from novice to knowledgeable using systematic quantitative literature reviews. *Studies in Higher Education* 40:1756-1769

Pinder AM, Halse SA, McRae JM, Shiel RJ 2005. Occurence of aquatic invertebrates of the wheatbelt region of Western Australia in relation to salinity. *Hydrobiologia* 543:1-24

Prahalad V, Pearson J 2013. Southern Tasmanian coastal saltmarsh futures: A preliminary strategic assessment [contract report]. Natural Resource Management South. Hobart, Tasmania.

Prahalad VN, Kirkpatrick JB, Mount RE 2012. Tasmanian coastal saltmarsh community transitions associated with climate change and relative sea level rise 1975–2009. *Australian Journal of Botany* 59:741-748

R Core Team 2019. R: A language and environment for statistical computing R version 3.5.3 (Great Truth) ed. Vienna, Austria.

Ramírez A, Gutiérrez-Fonseca PE 2014. Functional feeding groups of aquatic insect families in Latin America: A critical analysis and review of existing literature. *Revista de Biología Tropical* 62:155-167

Rapport DJ 1999. Epidemiology and ecosystem health: Natural bridges. *Ecosystem Health* 5:174-180

Reiskind MH, Lounibos LP 2009. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Medical and Veterinary Entomology* 23:62-68

Richardson AMM, Swain R, Wong V 1998. Relationship between the crustacean and molluscan assemblages of Tasmanian saltmarshes and the vegetation and soil conditions. *Marine and Freshwater Research* 49:785-799

Ritchie SA 1994. Spatial stability of *Aedes vigilax* (Diptera: Culicidae) eggshells in southeastern Queensland salt marshes. *Journal of Medical Entomology* 31:920-922

Ritchie, SA, and Addison DS 1992. Oviposition preferences of *Aedes taeniorhynchus* (Diptera: Culicidae) in Florida mangrove forests. *Environmental Entomology*. 21: 737-744

Robertson GJ, Doggett S, Seeman O, Russell RC, Clancy J, Haniotis J 2004. Ross River virus and its vectors in Sorell municipal area, south-eastern Tasmania, January to March 2002. *Communicable Disease Intellegence* 28:261-266

Rochlin I, James-Pirri MJ, Adamowicz SC, Wolfe RJ, Capotosto P, Dempsey ME, Iwanejko T, Ninivaggi DV 2012. Integrated marsh management (IMM): A new perspective on mosquito control and best management practices for salt marsh restoration. *Wetlands Ecology and Management* 20:219-232

Rogers K, Boon PI, Branigan S, Duke NC, Field CD, Fitzsimons JA, Kirkman H, Mackenzie JR, Saintilan N 2016. The state of legislation and policy protecting Australia's mangrove and salt marsh and their ecosystem services. *Marine Policy* 72:139-155

Roiz D, Ruiz S, Soriguer R, Figuerola J 2015. Landscape effects on the presence, abundance and diversity of mosquitoes in Mediterranean wetlands. *PLoS one* 10:e0128112

Rossi V, Benassi G, Belletti F, Menozzi P 2011. Colonization, population dynamics, predatory behaviour and cannibalism in *Heterocypris incongruens* (Crustacea: Ostracoda). *Journal of Limnology* 70:102-108

Rowbottom R, Carver S, Barmuta LA, Weinstein P, Allen GR 2017. Mosquito distribution in a saltmarsh: Determinants of eggs in a variable environment. *Journal of Vector Ecology* 42:161-170

Rowbottom R, Carver S, Barmuta LA, Weinstein P, Allen GR 2020. How do local differences in saltmarsh ecology influence disease vector mosquito populations? *Medical and Veterinary Entomology* DOI: 10.1111/mve.12433

Rowbottom R, Carver S, Barmuta LA, Weinstein P, Foo D, Allen GR 2015. Resource limitation, controphic ostracod density and larval mosquito development. *PLoS one* 10:e0142472

Russell RC 1993. Mosquitoes and mosquito-borne disease in southeastern Australia: A guide to the biology, relation to disease, surveillance, control and the identification of mosquitoes in southeastern Australia, New South Wales: Department of Medical Entomology, Westmead Hospital and Department of Medicine, pp 195

Russell RC 1995. Arboviruses and their vectors in Australia: An update on the ecology and epidemiology of some mosquito-borne arboviruses. *Review of Medical and Veterinary Entomology* 83:141-158

Russell RC 1998. Mosquito-borne arboviruses in Australia: The current scene and implications of climate change for human health. *International Journal for Parasitology* 28:955-969

Russell RC 2002. Ross river virus; ecology and distribution. *Annual Review of Entomology* 47:1-31

Russell RC, Cloonan MJ, Wells PJ, Vale TG 1991. Mosquito (Diptera: Culicidae) and arbovirus activity on the south coast of New South Wales, Australia, in 1985–1988. *Journal of Medical Entomology* 28:796-804

Russell RC, Dwyer DE 2000. Arboviruses associated with human disease in Australia. *Microbes and Infection* 2:1693-1704

Russell RC, Kay BH 2004. Medical entomology: Changes in the spectrum of mosquito-borne disease in Australia and other vector threats and risks, 1972-2004. *Australian Journal of Entomology* 43:271-282

Russell RC, Vale TG, Wells PJ, Cloonan MJ 1992. Monthly periodicity and abundance of mosquitoes (Diptera: Culicidae) near Batemans Bay on the south coast of New South Wales, 1985-1988. *Journal of the Australian Entomological Society* 31:281-288

Russell TL, Gatton ML, Ryan PA, Kay BH 2009a. Quality assurance of aerial applications of larvicides for mosquito control: Effects of granule and catch tray size on field monitoring programs. *Journal of Economic Entomology* 102:507-514

Russell TL, Kay BH, Skilleter GA 2009b. Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. *Aquatic Biology* 6:77-90

Ryan PA, Do KA, Kay BH 2000. Definition of Ross River virus vectors at Maroochy Shire, Australia. *Journal of Medical Entomology* 37:146-152

Ryan PA, Kay BH 1999. Vector competence of mosquitoes (Diptera: Culicidae) from Maroochy Shire, Australia, for Barmah Forest virus. *Journal of Medical Entomology* 36:856-860

Rydzanicz K, Kącki Z, Jawień P 2011. Environmental factors associated with the distribution of floodwater mosquito eggs in irrigated fields in Wrocław, Poland. *Journal of Vector Ecology* 36:332-342

Saffigna PG, Dale P 1999. Acid sulfate soils in intertidal mosquito breeding habitats and implications for habitat modification. *AGRIS* 15:520-525

Saintilan N 2009a. Australian saltmarsh ecology, Collingwood, Victoria: CSIRO Publishing, pp 236

Saintilan, N. 2009b. Biogeography of Australian saltmarsh plants. *Austral Ecology*. 34: 929-937

Saintilan N, Rogers K 2013. The significance and vulnerability of Australian saltmarshes: Implications for management in a changing climate. *Marine and Freshwater Research* 64:66-79

Schneider JR, Chadee DD, Mori A, Romero-Severson J, Severson DW 2011. Heritability and adaptive phenotypic plasticity of adult body size in the mosquito *Aedes aegypti* with implications for dengue vector competence. *Infection, Genetics and Evolution* 11:11-16

Shinkarenko L, Hulsman K, Mottram P, Dale P, Kay BH 1986. Reliability of using head capsule width and body length to identify larval instars of *Aedes vigilax* (Diptera: Culilcidae). *Australian Journal of Entomology* 25:37-40

Silberbush A, Tsurim I, Margalith Y, Blaustein L 2014a. Interactive effects of salinity and a predator on mosquito oviposition and larval performance. *Oecologia* 175:565-575

Silberbush A, Tsurim I, Rosen R, Margalith Y, Ovadia O 2014b. Species-specific non-physical interference competition among mosquito larvae. *PLoS one* 9:e88650

Sinclair P 1976. Notes on the biology of the salt-marsh mosquito, *Aedes vigilax* (Skuse) in south-east Queensland. *Queensland Naturalist* 21:134-139

Stav G, Blaustein L, Margalit Y 2005. Individual and interactive effects of a predator and controphic species on mosquito populations. *Ecological Applications* 15:587-598

Stephenson EB, Peel AJ, Reid SA, Jansen CC, McCallum H 2018. The non-human reservoirs of Ross River virus: A systematic review of the evidence. *Parasites & Vectors* 11: 188; DOI: 10.1186/s13071-018-2733-8

Stone CM, Jackson BT, Foster WA 2012. Effects of plant-community composition on the vectorial capacity and fitness of the malaria mosquito *Anopheles gambiae*. *American Journal of Tropical Medicine and Hygiene* 87:727-736

Tall JA, Gatton ML, Tong S 2014. Ross River virus disease activity associated with naturally occurring nontidal flood events in Australia: A systematic review. *Journal of Medical Entomology* 51:1097-1108

Talley DM, Talley TS 2008. Salinity, In: Jørgensen, SE & Fath, BD, *Encyclopedia of Ecology* Oxford: Academic Press, pp 3127-3132

Telang A, Li Y, Noriega FG, Brown MR 2006. Effects of larval nutrition on the endocrinology of mosquito egg development. *Journal of Experimental Biology* 209:645-655

Tesh RB, McLean RG, Shroyer DA, Calisher CH, Rosen L 1981. Ross River virus (Togaviridae: Alphavirus) infection (epidemic polyarthritis) in American Samoa. *Transactions of the Royal Society for Tropical Medicine and Hygiene* 75:426-431

Tilman D 1982. Resource competition and community structure, (Monographs in population biology; 17), Princeton, New Jersey: Princeton University Press, pp 296

Tong S, Hu W 2002. Different responses of Ross River virus to climate variability between coastline and inland cities in Queensland, Australia. *Occupational and Environmental Medicine* 59:739-744

Tong S, Hu W, Nicholls N, Dale P, MacKenzie JS, Patz J, McMichael AJ 2005. Climatic, high tide and vector variables and the transmission of Ross River virus. *Internal Medicine Journal* 35:677-680

Tun-Lin W, Burkot TR, Kay BH 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. *Medical and Veterinary Entomology* 14:31-37

Turner PA 2002. Relationship between age and colour of hatched eggshells of *Ochlerotatus vigilax* (Skuse) (Diptera: Culicidae) on an Australian saltmarsh. *Australian Journal of Entomology* 41:324-328

Turner PA, Streever WJ 1997. The relationship between the density of *Aedes vigilax* (Diptera: Culicidae) eggshells and environmental factors on Kooragang Island, New South Wales, Australia. *Journal of the American Mosquito Control Association* 13:361-367

Turner PA, Streever WJ 1999. Changes in productivity of the saltmarsh mosquito, *Aedes vigilax* (Diptera: Culicidae), and vegetation cover following culvert removal. *Australian Journal of Ecology* 24:240-248

Vale TG, Dowling ML, Cloonan MJ 1992. Infection and multiplication of Ross River virus in the mosquito vector *Aedes vigilax* (Skuse). *Australian Journal of Zoology* 40:35-41

van den Hurk AF, Craig SB, Tulsiani SM, Jansen CC 2010. Emerging tropical diseases in Australia. Part 4. Mosquitoborne diseases. *Annals of Tropical Medicine and Parasitology* 104:623-640

van den Hurk AF, Hall-Mendelin S, Johansen CA, Warrilow D, Ritchie SA 2012. Evolution of mosquito-based arbovirus surveillance systems in Australia. *Journal of Biomedicine and Biotechnology* DOI: 10.1155/2012/325659

van Schie C. 2006. The salinity tolerance of *Culex australicus* and *Aedes camptorhynchus* from different regions of south-western Australia. Honours, University of Western Australia.

van Schie C, Spafford H, Carver S, Weinstein P 2009. Salinity tolerance of *Aedes camptorhynchus* (Diptera: Culicidae) from two regions in southwestern Australia. *Australian Journal of Entomology* 48:293-299

Vonesh JR, Blaustein L 2010. Predator-induced shifts in mosquito oviposition site selection: A meta-analysis and implications for vector control. *Israel Journal of Ecology & Evolution* 56:263-279

Wachira SW, Ndung'U M, Njagi PGN, Hassanali A 2010. Comparative responses of ovipositing *Anopheles gambiae* and *Culex quinquefasciatus* females to the presence of Culex egg rafts and larvae. *Medical and Veterinary Entomology* 24:369-374

Walker LJ, Selvey LA, Jardine A, Johansen CA, Lindsay MDA 2018. Mosquito and virus surveillance as a predictor of human Ross River virus infection in south-west Western Australia: How useful is it? *American Journal of Tropical Medicine and Hygiene* 99:1066-1073

Walsh R. 2015. Personnal communication: *Australian Waterlife,* Aquatic Micro-invertebrate Ecologist, Freshwater Ecology & Limnology, www.australianwaterlife.com.au.

Weaver SC, Reisen WK 2010. Present and future arboviral threats. *Antiviral Research* 85:328-345

Webb CE 2013 Managing mosquitoes in coastal wetlands, Chapter 3.6. In: Swapan P, *Workbook for managing urban wetlands in Australia,* , Sydney Olympic Park Authority, Australia, NSW, pp 321-340

Webb CE, Russell RC 1999. Towards management of mosquitoes at Homebush Bay, Sydney, Australia. I. Seasonal activity and relative abundance of adults of *Aedes vigilax, Culex sitiens,* and other salt-marsh species, 1993-94 through 1997-98. *Journal of the American Mosquito Control Association* 15:242-249

Webb G, Miller P, Peters B 2012. Pyripoxyfen for the control of Australian salt-marsh mosquito, *Aedes vigilax*. *Journal of the American Mosquito Control Association* 28:50-52

Weinstein JE 1996. Anthropogenic impacts on salt marshes - A review. In: Vernberg, FJ, Vernberg, WB & Siewicki, T, *Urbanisation of Southeastern Estuaries*, University of South Carolina Press, Columbia, South Carolina, pp 135-170

Weinstein P 1997. An ecological approach to public health intervention: Ross River virus in Australia. *Environmental Health Perspectives* 105:364-366

Werner AK, Goater S, Carver S, Robertson G, Allen GR 2012. Environmental drivers of Ross River virus in southeastern Tasmania, Australia: Towards strengthening public health interventions. *Epidemiology and Infection* 140:359-371

Williams CR, Bader CA, Williams SR, Whelan PI 2012. Adult mosquito trap sensitivity for detecting exotic mosquito incursions and eradication: A study using EVS traps and the Australian southern saltmarsh mosquito, *Aedes camptorhynchus*. *Journal of Vector Ecology* 37:110-116

Williams CR, Rau G 2011. Growth and development performance of the ubiquitous urban mosquito *Aedes notoscriptus* (Diptera: Culicidae) in Australia varies with water type and temperature. *Australian Journal of Entomology* 50:195-199

Williams CR, Williams SR, Nicholson J, Little SM, Riordan J, Fricker SR, Kokkinn MJ 2009a. Diversity and seasonal succession of coastal mosquitoes (Diptera: Culicidae) in the northern Adelaide region of South Australia. *Australian Journal of Entomology* 48:107-112

Williams DD 1997. Temporary ponds and their invertebrate communities. *Aquatic Conservation: Marine and Freshwater Ecosystems* 7:105-117

Williams SR, Kokkinn MJ, Fricker SR, Schneemilch MJ, Humphrey MJ, Williams CR 2009b. Mosquitoes (Diptera: Culicidae) of the Spencer Gulf Coast of South Australia. *Transactions of the Royal Society of South Australia* 133:51-56

Wisz SM, Pottier J, Kessling WD, Pellissier L, Lenoir J, Damgaard CF, Dormann CF, Forchhammer MC, Grytnes JA, Guisan A, Heikkinen RK, Høye TT, Kühn I, Luoto M, Maiorano L, Nilsson MC, Normand S, Őckinger E, Schmidt NM, Termansen M,

Timmermann A, Wardie DA, Aastrup P, Svenning JC 2013. The role of biotic interactions in shaping distributions and realised assemblages of species: Implications for species distribution modelling. *Biological Reviews* 88:15-30

Woodruff R, Guest C, Garner G, Becker N, Lindsay MF 2003. Weather and climate as early warning system indicators for epidemics of Ross River virus: A case study in south-west Western Australia. *Epidemiology* 14:S94

Woodruff RE, Bambrik H 2008. Climate change impacts on the burden of Ross River virus disease. *Garnaut Climate Change Review* 1–15

Yang G-J, Brook BW, Bradshaw CJA 2009. Predicting the timing and magnitude of tropical mosquito population peaks for maximizing control efficiency. *PLoS Neglected Tropical Diseases* 3:e385

Yang GJ, Brook BW, Whelan PI, Cleland S, Bradshaw CJA 2008. Endogenous and exogenous factors controlling temporal abundance patterns of tropical mosquitoes. *Ecological Applications* 18:2028-2040

Yoshioka M, Couret J, Kim F, McMillan J, Burkot TR, Dotson EM, Kitron U, Vazquez-Prokopec GM 2012. Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). *Parasites & Vectors* 5: 255; DOI: 10.1186/1756-3305-5-225

Yu W, Dale P, Turner L, Tong S 2014. Projecting the impact of climate change on the transmission of Ross River virus: Methodological challenges and research needs. *Epidemiology & Infection* 142:2013-2023

Zedler JB, Nelson, P. and Adam, P. 2010. Plant community organization in New South Wales saltmarshes: Species mosaics and potential causes. *Wetlands Australia* 14:1-18

Zhang Y, Bi P, Hiller JE 2008. Climate change and the transmission of vector-borne diseases: A review. *Asia-Pacific Journal of Public Health* 20:64-76

Appendices

Appendix I - Saltmarshes

Appendix I: Primrose Sands Saltmarsh

Appendix I: Cambridge Saltmarsh

Appendix I: Richmond Saltmarsh

Appendix II – Published chapters

It is the following published article: Rowbottom, R., Carver, S., Barmuta, L. A., Weinstein, P., Allen, G. R., 2020, How do local differences in saltmarsh ecology influence disease vector mosquito populations?, Medical and veterinary entomology, 34(3), 279-290

Mosquito distribution in a saltmarsh: determinants of eggs in a variable environment

Raylea Rowbottom[1]✉, Scott Carver[2], Leon A. Barmuta[2], Philip Weinstein[3], and Geoff R. Allen[1]

[1]*School of Land and Food/TIA, University of Tasmania, Hobart, Australia raylea.rowbottom@utas.edu.au*
[2]*School of Biological Sciences, University of Tasmania, Hobart, Australia*
[3]*School of Biological Sciences, University of Adelaide, Adelaide, Australia*

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ABSTRACT: Two saltmarsh mosquitoes dominate the transmission of Ross River virus (RRV, Togoviridae: Alphavirus), one of Australia's most prominent mosquito-borne diseases. Ecologically, saltmarshes vary in their structure, including habitat types, hydrological regimes, and diversity of aquatic fauna, all of which drive mosquito oviposition behavior. Understanding the distribution of vector mosquitoes within saltmarshes can inform early warning systems, surveillance, and management of vector populations. The aim of this study was to identify the distribution of *Ae. camptorhynchus*, a known vector for RRV, across a saltmarsh and investigate the influence that other invertebrate assemblage might have on *Ae. camptorhynchus* egg dispersal. We demonstrate that vegetation is a strong indicator for *Ae. camptorhynchus* egg distribution, and this was not correlated with elevation or other invertebrates located at this saltmarsh. Also, habitats within this marsh are less frequently inundated, resulting in dryer conditions. We conclude that this information can be applied in vector surveillance and monitoring of temperate saltmarsh environments and also provides a baseline for future investigations into understanding mosquito vector habitat requirements. ***Journal of Vector Ecology* 42 (1): 161-170. 2017.**

Keyword Index: Ross River virus, *Aedes camptorhynchus,* oviposition, saltmarsh, *Sarcocornia,* ostracods.

INTRODUCTION

Saltmarsh mosquitoes include major vectors of disease worldwide. This is particularly notable across the continent of Australia, where two saltmarsh mosquito species dominate transmission of Ross River virus (RRV), Australia's most prominent mosquito-borne disease. Accordingly, these habitats are a focus of major vector control and surveillance efforts targeting egg and larval stages of mosquito development. However, there is little knowledge about egg distribution of target mosquitoes within the complexity of saltmarsh environments. This knowledge is informative for determining potentially preferred oviposition habitats or areas of optimal egg survival, and thus can support control efforts, supplement early warning systems, and assist in more effective surveillance and management of vector mosquito populations. It can also contribute fundamental information on the natural history of this ecologically important environment.

Saltmarshes vary in their ecological structure, including habitat types, hydrological regimes, and diversity of aquatic fauna, all of which drive mosquito oviposition behavior (Adam 2002, McCall and Pennings 2012). Across Australia, saltmarsh vegetation is unique in the overlap of mangroves and halophytic succulent plants, grasses, and sedges between the tropics and cooler temperate regions, with mangroves being absent from Tasmania (Laegdsgaard 2006, Saintilan and Rogers 2013). This provides a mosaic of habitats and environmental conditions that can be utilized by mosquito vectors. Females of aedine mosquito species oviposit eggs on damp substrate or regions of the saltmarsh where water periodically occurs from tides or rainfall (Dale and Knight 2008). For example, several studies have associated *Aedes vigilax* (Skuse) with oviposition regions near drainage channels that are subject to tidal inundations (Gislason and Russell 1997, Jacups et al. 2009). Similarly, aquatic interactions between mosquitoes and predator/competitors within saltmarshes can also impact mosquito oviposition, larval development, survival, adult size (Mokany and Shine 2003, Knight et al. 2004, Vonesh and Blaustein 2010), and potentially egg survival. Consequently, these effects have potential to change mosquito fitness and vector capacity (Alto et al. 2005, Bara et al. 2015).

In southern Tasmania, *Ae. camptorhynchus* (Thomson) (Diptera: Culicidae) is a recognized vector of RRV (Harley et al. 2001, Russell 2002, Cutcher et al. 2017). This halotolerant species is the dominant mosquito in saltmarsh habitats and notoriously bites humans (Barton et al. 2004, Williams et al. 2009), with populations in southern Tasmania most abundant from Spring to Autumn (October - April) (Werner et al. 2012). The presence of other fauna (microcrustaceans) was formally proposed to have a role in regulating *Ae. camptorhynchus* abundance within saltmarshes (Carver et al. 2011). However, more recent laboratory evidence suggests this form of competition may have limited effects on *Ae. camptorhynchus* development (Rowbottom et al. 2015). Currently, little is understood about the distribution of *Ae. camptorhynchus* or its potential competitors within the saltmarsh.

At present, larval abundance of *Ae. camptorhynchus*is is known to be associated with samphire (*Sarcocornia sp.*) habitats (Carver et al. 2011). Similar affiliations with samphire (*Sarcocornia quinqueflora* (Scott)) and salt water couch (*Sporobolus virginicus* (Kunth)) have been identified for populations of *Ae. vigilax,* an ecologically similar species, although more prevalent in warmer Australian climates (Dale et al. 2008). However, other environmental conditions, such as hydrology, and/or competition with other aquatic fauna can also alter mosquito distribution. Therefore, the aims of this study were to (1) determine saltmarsh

habitats in which *Ae. camptorhynchus* eggs are most abundant, (2) investigate environmental conditions that may favor mosquito abundance within saltmarsh habitats, and (3) examine the use of the same sites by ostracods, the dominant microcrustacean species and putative competitor of *Ae. camptorhynchus* in this saltmarsh, and other invertebrates.

Given the spatial occurrence of both *Ae. vigilax* and *Ae. camptorhynchus* throughout mainland Australia, we expect to observe greater egg distribution among samphire (*Sarcocornia sp.*). We also expect that hydrological influences, such as tidal inundations and heavy rainfall, would affect egg dispersal and abundance. Considering the absence of significant predator species in this salt marsh, we do not expect the presence of other invertebrates to influence egg distribution, but given the dynamic nature of salt marsh habitats, we discuss other mechanisms that may have a role in egg distribution.

MATERIALS AND METHODS

Field sampling

This study was conducted at a saltmarsh in Primrose Sands (147° 39′ E, 42° 52′ S) in southern Tasmania (Figure 1). This saltmarsh is known for its seasonally large populations of *Ae. camptorhynchus* (Robertson et al. 2004, Carver et al. 2011). Overall, this marsh ranges in elevation from 0.38 m to 3.2 m with a bank that reaches above 1.5 m along the Carlton River, a saline and tidal area of the river near its terminus with the ocean. Cores of vegetation and soil were collected from the saltmarsh to evaluate egg distribution of *Ae. camptorhynchus* and other invertebrates, including dormant microcrustaceans. We sampled the saltmarsh by categorizing the environment into four dominant habitat types; 1) bare soil (BS), 2) runnels (human-made from early 1950s) (R), 3) samphire/beaded glasswort (*Sarcocornia quinqueflora*) (S), and 4) shrubby glasswort (*Tecticornia arbuscula* (Sheph and Wilson)) (SG). The saltmarsh was longitudinally divided into three 1-km sections that allowed for sampling from the river side of the marsh, center marsh, and the embankment. Each transect was separated by 47 m and followed a bearing of 030 along the main edge of the Carlton River. To make sure each habitat type was sampled evenly across the saltmarsh, all three transects were divided into 200 m quarters in which all four dominant habitat types were sampled (Figure 1). Field samples were collected over the peak season of RRV and mosquito abundance in Tasmania (October-March) at times when an abundance of larvae and pupae were observed. These collections also coincided with an absence of excessive rainfall and tidal inundations for approximately one week to allow enough time for adult emergence, mating, feeding, and oviposition to occur (Gillett 1971).

There were three sampling periods, October, December, and February 2012-2013. To maintain random sampling of habitat types within each quarter of each transect, a random starting point (distance in meters from the beginning of each transect) was allocated for each transect. From the start of each transect, increments of 10 m were travelled at which point a core sample was taken, providing there was a suitable unsampled habitat type. If not, the distance was travelled again until all four habitat types were sampled within each quarter. At each consecutive sampling period, December and February, the starting point of sampling from each transect line was taken at 6 m and 32 m, respectively. This was to ensure core samples were not from the same locations as the previous sampling periods. Stainless steel circular cores (589 cm^3) were used for all core samples. Once extracted, each core was placed in 20 cm round plastic containers for transport and laboratory inundations. The drainage systems, due to their positioning in the marsh and possibility of being unsampled in the above system, were individually targeted within each quarter.

At each sampling location, for each habitat type, a GPS location was recorded as well as temperature using a hand-held logger. In addition, the soil water content was measured for each habitat type for each transect by taking a 141 cm^3 soil core sample. These were placed in a 21 x 15 cm ziplock bag and the wet weight recorded. To assess the dry weight of these soil cores, each sample was removed from the zip lock bags and placed in 25 x 14 cm foil trays and dried in an oven (Qualtex 68 R4) at 105° C for approximately two weeks. At the completion of the drying period all cores were placed back in their ziplock bags and re-weighed.

Environmental data

Rainfall, temperature, and tide height were all recorded over the collection period. Daily maximum and minimum temperature was recorded using temperature loggers (Thermocron TC, On Solution Pty. Ltd) set to record every hour. These were positioned at the saltmarsh 1 m above the ground in Stevenson screens and data were extracted monthly. Rainfall was measured from rain gauges situated in the center of the marsh that were emptied weekly. Tidal height was obtained from titanium HOBO water level loggers (Onset Solutions, U20-001-04 Ti) placed in stilling wells. Stilling wells consisted of 25 cm long, 8 cm diameter PVC pipes with sealed caps on either end. Seven horizontal incisions were cut at the base of the pipes to allow for water flow/drainage. Inside the pipes sat the titanium HOBO water level loggers which were encased in PVC tube sitting on a bolt. Each stilling well was attached to a star picket to prevent damage and allow accuracy of readings.

Laboratory conditions

To assess which habitat type was most likely for *Ae. camptorhynchus* egg distribution, the top 10 cm of soil from each core was cut from each sample and placed back into the plastic containers (along with any plant material). All cores were kept at 10° C for 48 h then placed in a temperature controlled unit (Andrew Thorn Limited Qualtex 68 R4) set to 20 ± 0.3° C 12:12 day: night cycle, and inundated with 250 ml saline water at 35 ppt ("Red Sea Salts"; at 35 ppt elements are: 8.2-8.4 pH, 7.8-8.3 Alk (dKH), 420-440 Ca (mg/liter), 1250-1310 Mg (mg/liter), and 380-400 K (mg/liter)). Cores were inundated to promote hatching of dormant eggs and to examine the species assemblage of the different habitat types as a means of assessing the relationship between mosquito and macroinvertebrate egg distribution (Banerjee et al. 2010). The temperature chosen for laboratory conditions was based on the average spring/summer daily temperatures of water bodies in the field near Hobart (Rowbottom, unpublished data).

All cores were monitored daily to make sure they remained submerged, with water being replaced *ad-libitum*. To prevent the water going stagnant, a 19 gauge 1½ inch hypodermic needle (Neolus Terumo needles 1938) connected to one of two

Resun AC4000 Air Pumps using 8 mm plastic PVC hosing was placed inside each soil core container. All cores remained wet for five days at which time all the water was strained through a 150-micron sieve (99μm) so that invertebrate emergence could be scored. The core was then placed back in the temperature cabinet and left dry for seven days prior to a second inundation following the same procedure. Two wetting cycles were used to simulate environmental inundations from rainfall or tide and to allow for installment hatching (Bader and Williams 2011). All cores were randomly placed within the cabinet for both wetting cycles. All culicids were identified to species, with other invertebrates identified to order.

Data analysis

To identify *Ae. camptorhynchus* egg distribution, we evaluated how extrinsic components of the saltmarsh affected mosquito abundance. The bayesian model, described below, was the preferred analysis for all response variables because it is able to determine coefficients in mixed models with more accuracy (Gelman and Hill 2006) and the Poisson error distribution was most appropriate to handle over-dispersion given the use of count data.

Mosquito egg distribution was evaluated using Bayesian regression with habitat and elevation as the fixed effects, with month as a linear and quadratic term. Y_i was the average number of emerged mosquitoes within each replicate $i = 1,\ldots,n_i$. The distribution of eggs among replicates was assumed to have a Poisson distribution with parameter π_i:

$$Yi|\pi i \sim Poisson\ (\pi i)$$

where π_i is the modelled egg distribution of replicate *i*. We modelled the egg density, π_i, based on the effects of environmental variables,

$$\pi i = \alpha i + \beta i x i$$

where α and β are the model intercept and slope, respectively, for replicate *i* and x was the assigned environmental variable for replicate *i*. Prior distributions for all model parameters in the hierarchy were given with the goal of providing conjugate priors that contain little to no influence on the posterior distributions of all the model parameters. We assumed normal prior distributions on slopes, α, and intercepts, β, with mean μ and variance σ^2:

$$\alpha \sim \text{Normal}(\mu_\alpha, \sigma^2_\alpha)$$
$$\beta \sim \text{Normal}(\mu_\beta, \sigma^2_\beta)$$

For the variance parameters, σ^2, we determined and utilized non-informative uniform prior hyperparameter distributions, specified as σ^2~Uniform (0, 100), which was used across all models. Models were fitted in R (v 3.0.3) (Team 2016) using the 'MCMCglmm' package, with MCMC chains run for 13,000 iterations after a burn-in period of 1,000 iterations, ensuring convergence of model parameters, assessed following Gelman and Hill (2006). We summarized posterior distributions of model coefficients, β, by the Bayesian median and 95% credible intervals and MCMC simulated *P*-values. The effect of extrinsic variables on ostracod density and other invertebrate assemblage was also assessed using the above method.

RESULTS

Sampling covered the primary months when mosquito presence was expected to be observed in southern Tasmania. During this time, weather conditions remained constant with monthly daily maximum temperatures averaging 33.4° C (Table 1). There were no tidal events that resulted in the inundation of the entire saltmarsh, with the highest recorded tide reaching 1.84 m (Table 1).

The water content of soil cores varied between habitat types of the Primrose saltmarsh (26% to 55%; samphire and runnels, respectively). Runnels had the greatest water content followed by bare soil with samphire and shrubby glasswort having the least water content (Figure 2, Table 2). There was no association with water content and elevation. The water content of soil samples increased with each consecutive month of sampling (Table 2).

A total of 12,265 invertebrates emerged and was counted from the soil cores throughout the study period; of these, 3.6% (438) were *Ae. camptorhynchus* and 7.7% (947) were ostracods. A large constituent of the remaining invertebrates was a ubiquitous Copepoda (Table 3) with 79% (9688) individuals identified, however, both copepods and other invertebrates did not influence the distribution *Ae camptorhynchus* eggs (Table 4).

Mosquitoes identified from the samples were exclusively *Ae. camptorhynchus* larvae. Overall, egg distribution of *Ae. camptorhynchus* was most abundant by habitat type with 67.1% of mosquitoes emerging from cores collected from samphire habitats (Figure 3, Table 5). Of the remaining habitats, 29.9% emerged from shrubby glasswort cores with only 1.4% and 1.6% from bare soil and runnels, respectively. In comparison, ostracod numbers dominated the bare soil and runnel samples with a total of 82.3%. Shrubby glasswort and samphire habitats consisted of only 5.9% and 11.8% of ostracods, respectively. However, both the sporadic distribution and extreme fluctuations of ostracods resulted in no significant habitat preference (Figure 3, Table 5). Despite the dominant habitat distribution of both ostracods and *Ae. camptorhynchus* being dissimilar, their most abundant samples were spatially similar within the marsh (Figure 4). There was no significant association between ostracod abundance and *Ae. camptorhynchus* abundance (Table 5). There was little evidence that month affected the number of mosquito eggs present (Table 5). Apparent temporal increases in mosquito numbers (51, 116, 271 mosquitoes for October, December, and February respectively; Figure 3) were, also, driven by high egg numbers in a small number of samples with observed mosquito numbers ranging between 1 and 132 from a single core. By comparison, ostracod numbers peaked earlier in the sampling season with a maximum of 369 ostracods observed in bare soil samples in October and 296 in December. By February, numbers of ostracods fell below 75 individuals per habitat type. However, month did not significantly affect ostracod densities (Figure 3, Table 5). The range of ostracods observed from a single core sample ranged between 1 and 230.

Table 1. In situ weather data for the Primrose Sands saltmarsh, Tasmania, over the collection period (October, 2012 – February, 2013). Rainfall, temperature, and tide height data include monthly ranges and averages ± SD. Missing data is due to local bushfires.

	Oct	Nov	Dec	Jan	Feb	Overall Range
Rainfall (mm)	0 - 12	0 – 21	0 – 6	0 – 2.5	0 – 6	0 – 21
Mean ± SD	4.25 ± 5.32	6.4 ± 9.45	2.5 ± 3	0.5 ± 1.12	3.33 ± 3.06	4.21 ± 5.41
Temperature (°C)	3 – 30	5 – 33	5.5 – 33	0	6.5 – 35.5	3 – 35.5
Mean ± SD	13.52 ± 7.04	16.68 ± 7.46	11.88 ± 11.58	0	13.70 ± 11.25	12.21 ± 10.05
Tide (m)	0.71 – 1.64	0.72 – 1.62	0.65 – 1.78	0.84 – 1.84	0.83 – 1.70	0.64 – 1.84
Mean ± SD	1.01 ± 0.21	0.97 ± 0.21	1.06 ± 0.25	1.09 ± 0.26	1.00 ± 0.21	1.03 ± 0.23

Table 2. Bayesian mixed-effects regression results showing the differences in water content for habitat type (bare soil, runnel, shrubby glasswort, samphire), elevation, and month of collection. Variables with significantly different water content are in bold. Credible intervals are 95%.

Variable	Coeff.	2.5 % CI	97.5 % CI	pMCMC
(intercept)	-0.716	-1.326	-0.097	0.030
Runnel	0.318	0.118	0.513	0.004
Samphire	-0.400	-0.613	-0.213	<0.001
Shrubby glasswort	-0.320	-0.506	-0.124	0.006
Elevation	-0.397	-1.125	0.380	0.326
Month.L	0.197	0.078	0.313	<0.001
Month.Q	-0.061	-0.193	0.055	0.312

L – linear and Q - quadratic.

DISCUSSION

Where a mosquito deposits eggs is an essential element in its life history, shaping individual fitness, development, and vectorial capacity (Bentley and Day 1989, Stone et al. 2012). Because of this, research into understanding the drivers that influence mosquito egg dispersal, especially of vector mosquitoes, is essential for mosquito management, control, and evaluating disease risk. However, globally these habitats are extremely variable both environmentally and ecologically (Laegdsgaard 2006), thus resulting in variability of mosquito egg distribution. For example, populations of *Ae. taeniorhynchus* in Florida are driven by substrate moisture and frequency of tidal inundations (Knight and Baker 1962, Ritchie and Addison 1992), whereas in Argentina, separate environmental factors drove abundance of two mosquito species in relation to their ovipositional strategies (Cardo et al. 2012).

Notably, the Australian coastline spans several degrees of latitude with saltmarsh and mangrove habitats being ecologically variable from the northern, tropical regions to the southern-most temperate regions (Adam 2002). Mosquito research in Australia has focused on vector mosquitoes that utilize these coastal saline environments as they produce the majority of endemic vector mosquitoes (Russell 1993, Russell 1995, Dale et al. 2008). When determining mosquito egg dispersal and oviposition locations within these environments, it may be difficult to disentangle oviposition site preferences from potential field predation and egg survival. However, *Aedes* species display less predator avoidance with oviposition which has been proposed to be due to their egg-laying strategy and egg longevity in the field (Bentley and Day 1989, Vonesh and Blaustein 2010). Given the variability in mosquito oviposition and environmental conditions, local studies of saltmarsh habitats and the mosquito vectors within these environments contribute to current and future vector management by explaining knowledge of the most productive habitat and environmental conditions for vectors to emerge from, and thereby target, for control.

We conducted preliminary investigations of a southern Tasmanian saltmarsh that is a known region of large populations of *Ae. camptorhynchus* (Robertson et al. 2004). This mosquito is a prominent vector for RRV in Australia and the only vector for the virus in Tasmania (McManus and Marshall 1986, McManus 1994). Several studies have identified environmental drivers of *Ae. camptorhynchus* populations (Dhileepan et al. 1997, Barton et al. 2004, Werner et al. 2012), but there are no empirical data on egg distribution or site selection cues. Therefore, understanding the dynamics of this saltmarsh and the components that drive egg distribution of *Ae. camptorhynchus* will be useful in the management, surveillance, and implementation of any future targeted mosquito control (Webb and Russell 1999, Griffin et al. 2010, Kay and Russell 2013).

We determined samphire (*S. quinqueflora*) to be a dominant habitat type for *Ae. camptorhynchus* egg distribution in the Primrose saltmarsh, which is consistent with our understanding of aedine mosquito oviposition (Sinclair 1976). In Australia, a large proportion of research for vectors of disease in saltmarshes has focused on *Ae. vigilax*, with vegetation, in particular *Sarcocornia*

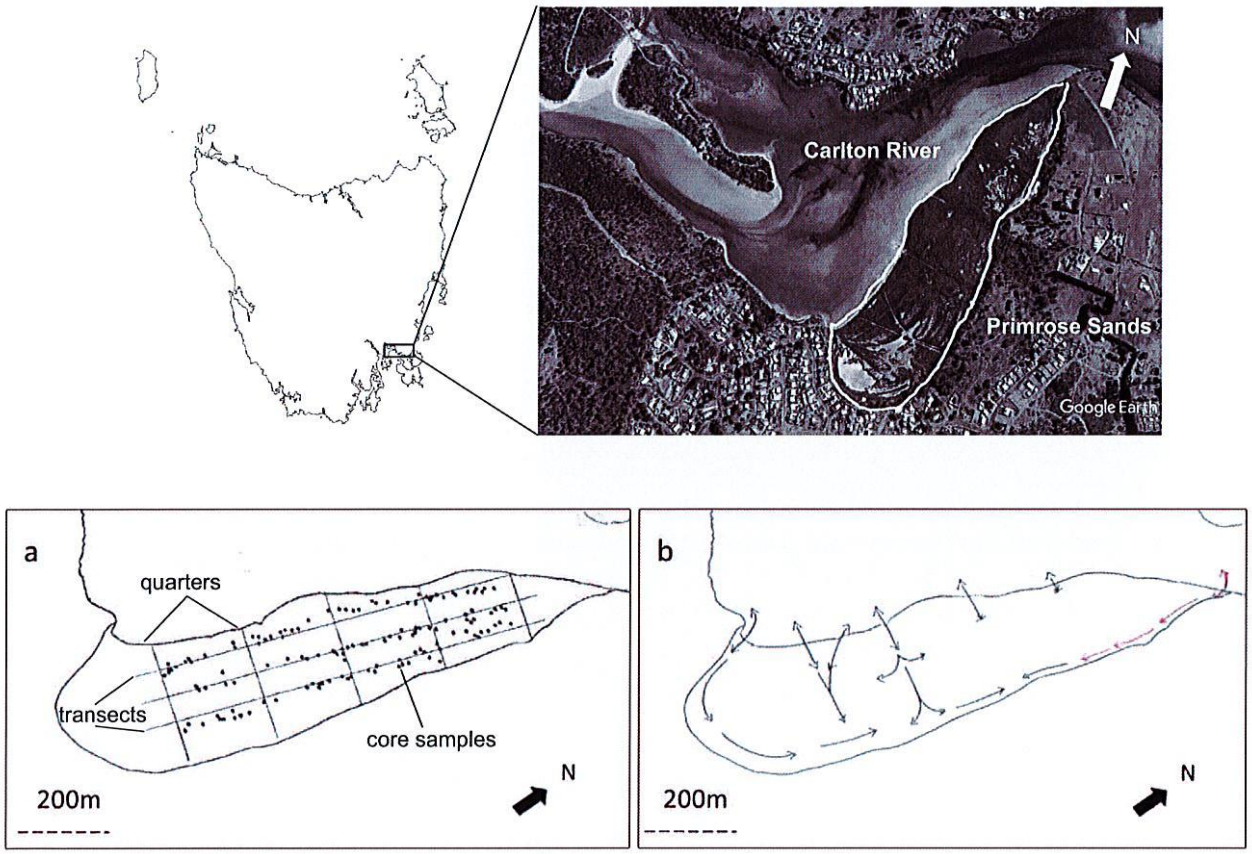

Figure 1. A map of the study area in southern Tasmania, displaying the saltmarsh region of Primrose Sands along the Carlton River. Sampling design including transect lines and core locations are presented in (a), and tidal hydrology (b). Detailed images of the saltmarsh are tilted to the east for aesthetics.

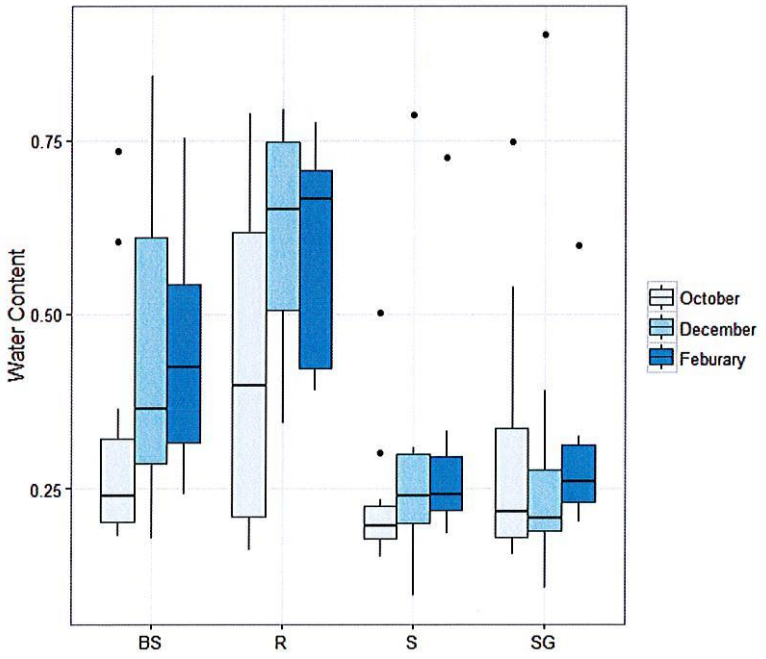

Figure 2. Percent water content from each habitat type for the three months of collection. Habitat types are as follows; (BS) bare soil, (R) runnel, (S) samphire and (SG) shrubby glasswort.

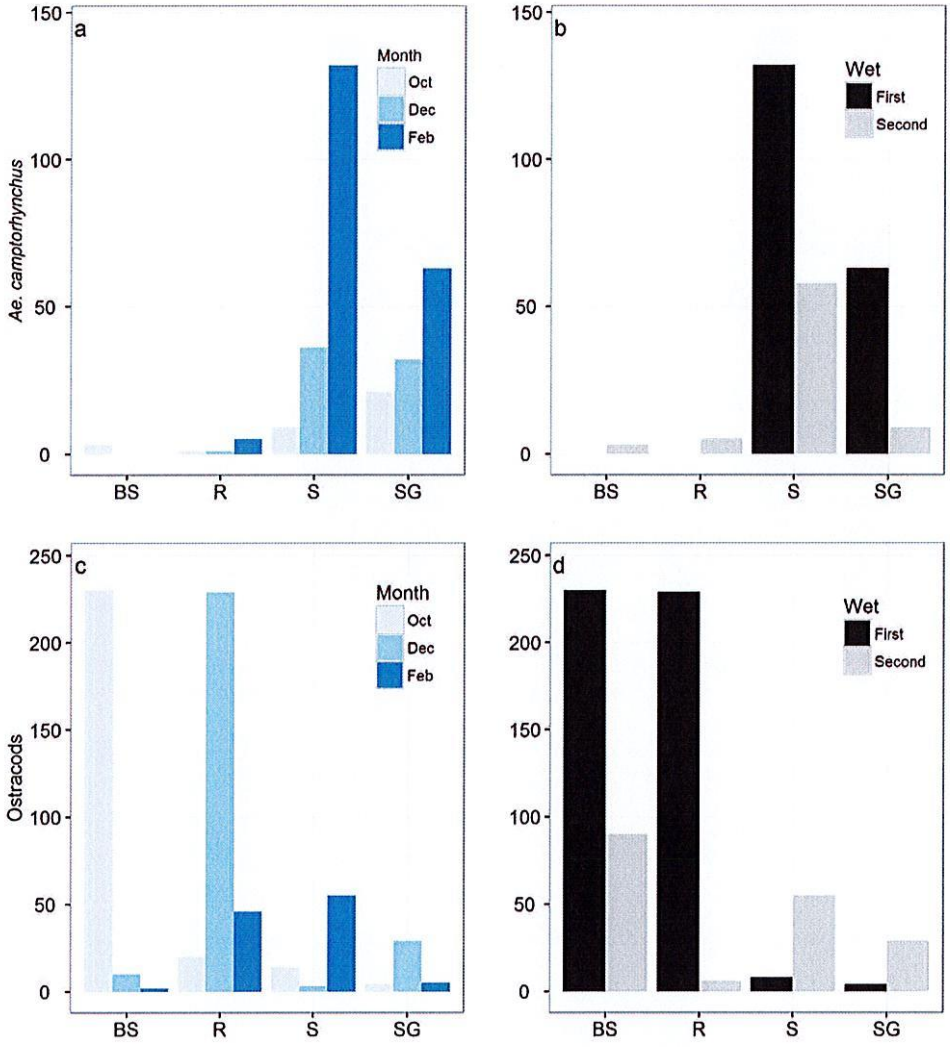

Figure 3. Total emergence Ae. camptorhynchus larva (a) and ostracods (c) from core samples for the three months of collection and total Ae. camptorhynchus (b) and ostracods (d) emergence from wetting cycles. Habitat types are as follows; (BS) bare soil, (R) runnel, (S) samphire and (SG) shrubby glasswort.

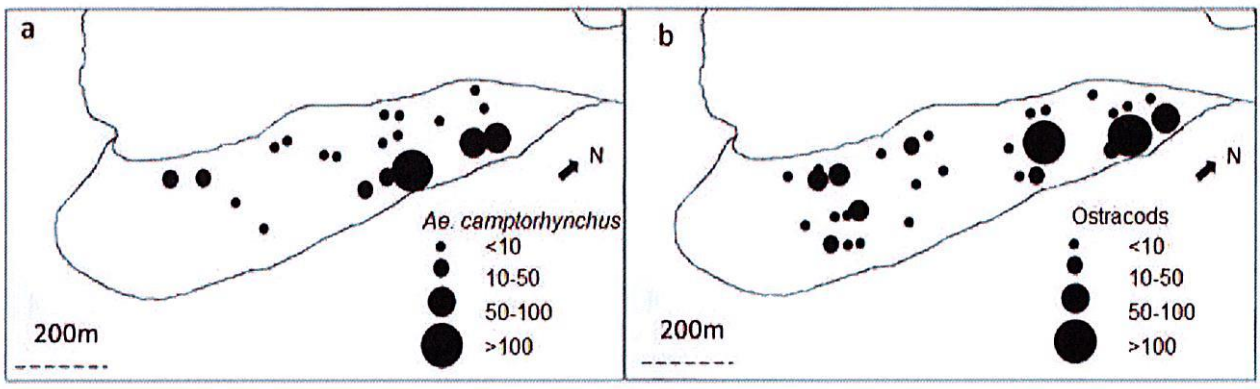

Figure 4. Collective density and distribution of *Ae. camptorhynchus* (a) and ostracods (b) from cores collected over the study period.

Table 3. A complete list of the remaining taxa identified from each habitat type in the core samples from the collection months.

	Copepods	Diptera	Gastropod	Collembola	Coleoptera	Acari	Amphipod	Oligochaetes	Unidentified
October									
Bare soil	1,509	9	0	0	6	4	1	0	2
Runnel	1,838	16	4	0	0	1	0	6	1
Shrubby glasswort	1,177	42	3	1	9	15	1	66	7
Samphire	2,527	78	1	2	3	29	0	47	29
December									
Bare soil	183	5	0	3	1	15	0	2	0
Runnel	315	30	1	1	0	10	0	0	0
Shrubby glasswort	367	4	0	11	6	32	1	32	0
Samphire	327	70	1	2	1	32	0	95	2
February									
Bare soil	57	6	0	0	1	2	0	3	0
Runnel	193	14	0	0	1	2	0	1	1
Shrubby glasswort	784	78	0	1	4	9	3	82	12
Samphire	411	106	2	0	9	23	0	78	14

Table 4. Bayesian negative binomial results showing the effects of other invertebrates on *Ae. camptorhynchus* egg distribution. Variables that contributed to *Ae. camptorhynchus* densities are in bold. Credible intervals are 95%.

Variable	Coeff.	2.5% CI	97.5% CI	pMCMC
Copepoda	-0.006	-0.023	0.007	0.408
Chironomidae	0.132	-0.155	0.394	0.318
Amphipoda	-2.442	-8.531	3.175	0.396
Acari	0.257	-0.296	0.764	0.334
Coleoptera	0.660	-1.626	2.763	0.532
Collembola	-3.317	-7.764	0.505	0.090
Diptera	0.470	-0.453	1.390	0.304
Gastropoda	-2.939	-9.895	2.189	0.294
unidentified	0.364	-0.269	0.997	0.258

spp., being the dominant determinant of oviposition sites (Dale et al. 1986, Gislason and Russell 1997, Dale et al. 2008). Yet in this study, the distribution of *Ae. camptorhynchus* was not as extensive as the distribution of *S. quinqueflora*, suggesting that not all regions occupied by this habitat are suitable. Saltmarsh vegetation is characterized by the tidal frequency, salinity, and the influence of surrounding land use (Saintilan 2009a). Changes to these conditions, such as increases in sea level or increased nutrient input from surrounding regions, can drastically influence floral composition (Prahalad et al. 2012) and therefore mosquito presence. Thus, both soil moisture and elevation may be relevant factors contributing to egg distribution.

Generally, there are strong correlations among elevation, tide height, and soil moisture within saltmarshes (Saintilan 2009b, Prahalad et al. 2012), which also correspond to mosquito abundance (Dale et al. 2008, Jacups et al. 2009). Moisture is an essential component in saltmarsh mosquito egg conditioning (Knight and Baker 1962, Kay and Jorgensen 1986) and hatching (Andreadis 1990, Rydzanicz et al. 2011, Cardo et al. 2012) and many studies correlate *Aedes* spp. with wet, lower regions of saltmarshes that are periodically flooded by tide (De Little et al. 2009, Jacups et al. 2009, Cardo et al. 2012). We found that soil moisture was greatest in bare soil and runnel habitat than shrubby glasswort and samphire, but there was no relationship between soil moisture and elevation. *Aedes camptorhynchus* egg distribution within the Primrose saltmarsh was greatest in samphire and unrelated to elevation, showing oviposition preference for habitat containing the least soil moisture. While this result appears counterintuitive, two non-mutually exclusive causes may explain this result: periodic inundation (flooding) of the saltmarsh by (1) very high tides and (2) heavy rainfall. The Primrose saltmarsh has a distinct bank, approximately 1.8 m along the river front of the marsh, which the tide would need to exceed to infiltrate the marsh. Thus, when flooding of the saltmarsh does occur it tends to be widespread. Similarly, Kokkinn et al. (2009) in South Australia report one of the factors determining abundance of *Ae. camptorhynchus* to be maximum tide heights, with peaks in adult abundance occurring only after such events. Given the extent of egg viability for *Ae. camptorhynchus* (Bader and Williams 2011), and the stability of environmental conditions during this study, it

Table 5. Bayesian negative binomial results showing the effects of habitat type (bare soil, runnel, shrubby glasswort, samphire), are elevation, month of collection, and density of ostracods on Ae. camptorhynchus egg distribution. Effects of ostracod densities with *Ae.camptorhynchus* abundance shown as the final variable. Variables that contributed to *Ae. camptorhynchus* or ostracod densities are in bold. Credible intervals are 95%.

Variable	Coeff.	2.5 % CI	97.5 % CI	pMCMC	Coeff.	2.5 % CI	97.5 % CI	pMCMC
	Ae. camptorhynchus				Ostracods			
Runnel	-0.037	-5.222	4.604	0.982	0.107	-1.851	2.058	0.914
Samphire	4.801	0.931	9.488	**0.012**	-0.936	-2.877	1.051	0.356
Shrubby glasswort	1.882	-2.488	5.993	0.390	-0.940	-3.103	0.964	0.328
Elevation	4.112	-11.980	20.695	0.590	4.195	-3.773	12.425	0.312
Month. L	-1.230	-3.592	0.986	0.284	-1.098	-2.497	0.107	0.094
Month. Q	0.472	-2.023	2.844	0.732	-0.744	-1.954	0.476	0.248
Ostracods	-0.047	-0.162	0.030	0.330				

may be advantageous for egg distribution of *Ae. camptorhynchus* to occur in normally dryer, more protected habitats in the saltmarsh to benefit survival and development.

Aside from habitat preference, another element in mosquito egg distribution is the presence of other invertebrates, namely, competitors and predators. Avoidance of both competitors and predators has been identified among mosquitoes, however, this behavior is not ubiquitous (Vonesh and Blaustein 2010). Therefore, we sought to understand the distribution and abundance of a putative competitor, a micro-crustacean ostracod (Carver et al. 2011). Significant inputs of water into these habitats are associated with mass hatching of eggs (Carver et al. 2011) at this site. During this study, we discovered that ostracods in this system are widely distributed across the saltmarsh with greater abundance in wetter regions but no differentiation in habitat type, which could be a result of recruitment by tide or shifts of egg banks by heavy rainfall or tidal inundation (Boulton and Lloyd 1992). In addition, there was no evident relationship between ostracod presence and mosquito distribution, suggesting that their significance as an aquatic competitor on mosquito egg distribution choices is minimal, and potentially ostracods and *Ae. camptorhynchus* may occupy separate niches within the water bodies (Rowbottom et al. 2015). Furthermore, there was no evidence that the presence of any other invertebrates found in these soil cores had any importance on *Ae. camptorhynchus* oviposition. The apparent lack of oviposition avoidance of *Ae. camptorhynchus* may also be a result of the ability to lay eggs above the water surface (Vonesh and Blaustein 2010, Yoshioka et al. 2012, Gonzalez et al. 2016) where they are protected from aquatic fauna that may consume them.

The results of our study demonstrate that vegetation is a strong indicator for *Ae. camptorhynchus* egg distribution and that this is a dryer habitat within the saltmarsh. Also, it appears that *Ae. camptorhynchus* egg distribution is not influenced by elevation or other invertebrates, albeit a depauperate fauna, located at this saltmarsh. Given that this saltmarsh is surrounded by a singular uniform land type and that different surrounding land use has been associated with changes in mosquito productivity (Claflin and Webb 2016), it would be informative to compare mosquito abundance with other local saltmarshes with a diversity of surrounding land types.

Knowledge of egg distribution and vector emergence patterns has many applications for future management. At present, there is little management or surveillance of mosquito populations within Tasmania. In fact, there is little empirical data on the spatial distribution of this vector in the state (Lee 1949, Robertson et al. 2004). Considering that there are discrepancies with habitat types and egg densities within mangrove systems (Dale et al. 2014), and that oviposition preferences are highly complex, the information from this study will act as a guide for future mosquito surveillance and knowledge of habitats that are likely regions for mosquito vectors. This is particularly important given the projected increase in human proximity to saltmarsh and inland wetland regions where *Ae. camptorhynchus* populations are likely to be (Lyth and Holbrook 2015). Furthermore, current models used to predict outbreaks of RRV based on environmental data (Werner et al. 2012) can benefit from understanding the spatial distribution of vector eggs within saltmarsh habitats and how this translates to sea level rise and tidal inundations.

We conclude that although habitat preference of *Ae. camptorhynchus* may be like other *Aedes* spp., other environmental preferences such as soil moisture and tidal height are species specific and may vary in different spatial contexts. This study has management applications in that vector control efforts for *Ae. camptorhynchus* should consider samphire habitat, even at higher elevations in the saltmarsh and that mass saltmarsh inundation events may be the main driver of *Ae. camptorhynchus* abundance.

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REFERENCES CITED

Adam, P. 2002. Saltmarshes in a time of change. Environ. Conserv. 29: 39-61.

Alto, B.W., L.P. Lounibos, S. Higgs, and S.A. Juliano. 2005. Larval competition differentially affects arbovirus infection in *Aedes* mosquitoes. Ecology 86: 3279-3288.

Andreadis, T.G. 1990. Observations on installment egg hatching in the brown salt-marsh mosquito, *Aedes-cantator*. J. Am. Mosq. Contr. Assoc. 6: 727-729.

Bader, C.A. and C.R. Williams. 2011. Eggs of the Australian saltmarsh mosquito, *Aedes camptorhynchus*, survive for long periods and hatch in instalments: implications for biosecurity in New Zealand. Med. Vet. Entomol. 25: 70-76.

Banerjee, S., G. Aditya, N. Saha, and G.K. Saha. 2010. An assessment of macroinvertebrate assemblages in mosquito larval habitats—space and diversity relationship. Environ. Monit. Assess. 168: 597-611.

Bara, J.J., Z. Rapti, C.E. Cáceres, and E.J. Muturi. 2015. Effect of larval competition on extrinsic incubation period and vectorial capacity of *Aedes albopictus* for dengue virus. PLoS One 10: e0126703.

Barton, P.S., J.G. Aberton, and B.H. Kay. 2004. Spatial and temporal definition of *Ochlerotatus camptorhynchus* (Thomson) (Diptera: Culicidae) in the Gippsland Lakes system of eastern Victoria. Aust. J. Entomol. 43: 16-22.

Bentley, M.D. and J.F. Day. 1989. Chemical ecology and behavioral-aspects of mosquito oviposition. Annu. Rev. Entomol. 34: 401-421.

Boulton, A.J. and L.N. Lloyd. 1992. Flooding frequency and invertebrate emergence from dry floodplain sediments of the River Murray, Australia. Regul. Rivers: Res. Manag. 7: 137-151.

Cardo, M.V., D. Vezzani, and A.E. Carbajo. 2012. Oviposition strategies of temporary pool mosquitoes in relation to weather, tidal regime and land use in a temperate wetland. Bull. Entomol. Res. 102: 651-662.

Carver, S., S. Goater, G.R. Allen, R. Parr, E. Fearnley, and P. Weinstein. 2011. Relationships of the Ross River Virus (Togoviridae: *Alphavirus*) vecor, *Aedes camptorhynchus* (Thomson) (Diptera: Culicidae), to biotic and abiotic factors in saltmarshes of south-eastern Tasmania, Australia: a preliminary study. Aust. J. Entomol. 50: 344-355.

Claflin, S.B. and C.E. Webb. 2016. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. Wetlands Ecol. Manag. 1-14.

Cutcher, Z., E. Williamson, S.E. Lynch, S. Rowe, H.J. Clothier, and S.M. Firestone. 2017. Predictive modelling of Ross River virus notifications in southeastern Australia. Epidemiol. Infect. 145: 440-450.

Dale, P., J. Knight, and L. Griffin. 2014. Comparing *Aedes vigilax* eggshell densities in saltmarsh and mangrove systems with implications for management. Insects 5: 984.

Dale, P.E.R., K. Hulsman, D. Harrison, and B. Congdon. 1986. Distribution of the immature stages of *Aedes-vigilax* on a coastal salt-marsh in Southeast Queensland. Aust. J. Ecol. 11: 269-278.

Dale, P.E.R., J. Knight, B.H. Kay, H. Chapman, S.A. Ritchie, and M.D. Brown. 2008. Habitat characteristics and eggshell distribution of the salt marsh mosquito, *Aedes vigilax*, in marshes in subtropical eastern Australia. J. Insect Sci. 8: 1-8.

Dale, P.E.R. and J.M. Knight. 2008. Wetlands and mosquitoes: a review. Wetl. Ecol. Manag. 16: 255-276.

De Little, S.C., D.M. Bowman, P.I. Whelan, B.W. Brook, and C.J.A. Bradshaw. 2009. Quantifying the drivers of larval density patterns in two tropical mosquito species to maximize control efficiency. Environ. Entomol. 38: 1013-1021.

Dhileepan, K., C. Peters, and A. Porter. 1997. Prevalence of *Aedes camptorhynchus* (Thomson) (Diptera: Culicidae) and other mosquitoes in the eastern eoast of Victoria. Aust. J. Entomol. 36: 183-190.

Gelman, A. and J. Hill. 2006. *Data Analysis Using Regression and Multilevel/Hierarchial Models*. Cambridge University Press, NY. 625 pp.

Gillett, J.D. 1971. *Mosquitoes*. Weidenfeld and Nicolson, London. 214.

Gislason, G.M. and R.C. Russell. 1997. Oviposition sites of the saltmarsh mosquito, *Aedes vigilax* (Skuse) (Diptera: Culicidae), at Homebush Bay, Sydney, NSW - A preliminary investigation. Aust. J. Entomol. 36: 97-100.

Gonzalez, P.V., P.A.G. Audino, and H.M. Masuh. 2016. Oviposition behavior in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in response to the presence of heterospecific and conspecific larvae. J. Med. Entomol. 53: 268-272.

Griffin, L.F., J.M. Knight, and P.E.R. Dale. 2010. Identifying mosquito habitat microtopography in an Australian mangrove forest using LiDAR derived elevation data. Wetlands 30: 929-937.

Harley, D., A. Sleigh, and S. Ritchie. 2001. Ross River virus transmission, infection, and disease: a cross-disciplinary review. Clin. Microbiol. Rev. 14: 909-932.

Jacups, S.P., N. Kurucz, P.I. Whelan, and J.M. Carter. 2009. A

comparison of *Aedes vigilax* larval population densities and associated vegetation categories in a coastal wetland, Northern Territory, Australia. J. Vector Ecol. 34: 311-316.

Kay, B.H. and W.K. Jorgensen. 1986. Eggs of *Aedes vigilax* (Skuse) and their distribution on plants and soil in South-East Queensland saltmarsh. J. Aust. Entomol. Soc. 25: 267-272.

Kay, B.H. and R.C. Russell. 2013. *Mosquito Eradication: The Story of Killing Campto*. CSIRO Publishing, VIC. 280 pp.

Knight, K.L. and T.E. Baker. 1962. The role of the substrate moisture content in the selection of oviposition sites by *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.). Mosquito News 22: 247-254.

Knight, T.M., J.M. Chase, C.W. Goss, and J.J. Knight. 2004. Effects of interspecific competition, predation, and their interaction on survival and development time of immature *Anopheles quadrimaculatus*. J. Vector Ecol. 29: 277-284.

Kokkinn, M.J., D.J. Duval, and C.R. Williams. 2009. Modelling the ecology of the coastal mosquitoes *Aedes vigilax* and *Aedes camptorhynchus* at Port Pirie, South Australia. Med. Vet. Entomol. 23: 85-91.

Laegdsgaard, P. 2006. Ecology, disturbance and restoration of coastal saltmarsh in Australia: a review. Wetl. Ecol. Manag. 14: 379-399.

Lee, D.J. 1949. Mosquitoes (Diptera, Culicidae) recorded from Tasmania. In: *Records of the Queen Victoria Museum Launceston*. Launceston: Museum Committee Launceston City Council.

Lyth, A. and N.J. Holbrook. 2015. Assessing an indirect health implication of a changing climate: Ross River Virus in a temperate island state. Clim. Risk Manag. 10: 77-94.

McCall, B.D. and S.C. Pennings. 2012. Geographic variation in salt marsh structure and function. Oecologia 170: 777-787.

McManus, T. 1994. Ross River virus: a review based on Tasmanian experience. In: *Australian Institute of Environmental Health*. Tasmania.

McManus, T. and I.D. Marshall. 1986. The epidemology of Ross River virus in Tasmania. Arbovir. Res. Aust. 4: 127-131.

Mokany, A. and R. Shine. 2003. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. Austral. Ecol. 28: 33-37.

Prahalad, V.N., J.B. Kirkpatrick, and R.E. Mount. 2012. Tasmanian coastal saltmarsh community transitions associated with climate change and relative sea level rise 1975–2009. Aust. J. Bot. 59: 741-748.

Ritchie, S.A. and D.S. Addison. 1992. Oviposition preferences of *Aedes taeniorhynchus* (Diptera: Culicidae) in Florida mangrove forests. Environ. Entomol. 21: 737-744.

Robertson, G.J., S. Doggett, O. Seeman, R.C. Russell, J. Clancy, and J. Haniotis. 2004. Ross River virus and its vectors in Sorell municipal area, south-eastern Tasmania, January to March 2002. Commun. Dis. Intell. 28: 261-266.

Rowbottom, R., S. Carver, L.A. Barmuta, P. Weinstein, D. Foo, and G.R. Allen. 2015. Resource limitation, controphic ostracod density and larval mosquito development. PLoS One 10: e0142472.

Russell, R.C. 1993. *Mosquitoes and Mosquito-Borne Disease in Southeastern Australia*. Department of Medical Entomology, Westmead Hospital and Department of Medicine, Sydney, NSW.

Russell, R.C. 1995. Arboviruses and their vectors in Australia: an update on the ecology and epidemiology of some mosquito-borne arboviruses. Rev. Med. Vet. Entomol. 83: 141-158.

Russell, R.C. 2002. Ross River virus; ecology and distribution. Annu. Rev. Entomol. 47: 1-31.

Rydzanicz, K., Z. Kącki, and P. Jawień. 2011. Environmental factors associated with the distribution of floodwater mosquito eggs in irrigated fields in Wrocław, Poland. J. Vector Ecol. 36: 332-342.

Saintilan, N. 2009a. *Australian Saltmarsh Ecology*. CSIRO Publishing, VIC. 231.

Saintilan, N. 2009b. Biogeography of Australian saltmarsh plants. Austr. Ecol. 34: 929-937.

Saintilan, N., and K.R. Rogers. 2013. The significance and vulnerability of Australian saltmarshes: implications for management in a changing climate. Mar. Freshw. Res. 64: 66-79.

Sinclair, P. 1976. Notes on the biology of the saltmarsh mosquito *Aedes vigilax* (Skuse) in south-east Queensland. Queensl. Nat. 21: 134-139.

Stone, C.M., B.T. Jackson, and W.A. Foster. 2012. Effects of plant-community composition on the vectorial capacity and fitness of the malaria mosquito *Anopheles gambiae*. Am. J. Trop. Med. Hyg. 87: 727-736.

Vonesh, J.R. and L. Blaustein. 2010. Predator-induced shifts in mosquito oviposition site selection: A meta-analysis and implications for vector control. Israel J. Ecol. Evol. 56: 263-279.

Webb, C.E. and R.C. Russell. 1999. Towards management of mosquitoes at Homebush Bay, Sydney, Australia. I. Seasonal activity and relative abundance of adults of *Aedes vigilax*, *Culex sitiens*, and other salt-marsh species, 1993-94 through 1997-98. J. Am. Mosq. Contr. Assoc. 15: 242-249.

Werner, A.K., S. Goater, S. Carver, G. Robertson, and G.R. Allen. 2012. Environmental drivers of Ross River virus in southeastern Tasmania, Australia; towards strengthening public health interventions. Epidemiol. Infect. 140: 359-371.

Williams, C.R., S.R. Williams, J. Nicholson, S.M. Little, J. Riordan, S.R. Fricker, and M.J. Kokkinn. 2009. Diversity and seasonal succession of coastal mosquitoes (Diptera: Culicidae) in the northern Adelaide region of South Australia. Aust. J. Entomol. 48: 107-112.

Yoshioka, M., J. Couret, F. Kim, J. McMillan, T.R. Burkot, E.M. Dotson, U. Kitron, and G.M. Vazquez-Prokopec. 2012. Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). Parasit. Vectors 5: 225-236.

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RESEARCH ARTICLE

Resource Limitation, Controphic Ostracod Density and Larval Mosquito Development

Raylea Rowbottom[1]*, Scott Carver[2], Leon A. Barmuta[2], Philip Weinstein[3], Dahlia Foo[2], Geoff R. Allen[1]

1 School of Land and Food/TIA, University of Tasmania, Hobart, Australia, **2** School of Biological Sciences, University of Tasmania, Hobart, Australia, **3** School of Pharmacy and Medical Science, University of South Australia, Adelaide, Australia

* raylea.rowbottom@utas.edu.au

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Abstract

Aquatic environments can be restricted with the amount of available food resources especially with changes to both abiotic and biotic conditions. Mosquito larvae, in particular, are sensitive to changes in food resources. Resource limitation through inter-, and intra-specific competition among mosquitoes are known to affect both their development and survival. However, much less is understood about the effects of non-culicid controphic competitors (species that share the same trophic level). To address this knowledge gap, we investigated and compared mosquito larval development, survival and adult size in two experiments, one with different densities of non-culicid controphic conditions and the other with altered resource conditions. We used *Aedes camptorhynchus*, a salt marsh breeding mosquito and a prominent vector for Ross River virus in Australia. *Aedes camptorhynchus* usually has few competitors due to its halo-tolerance and distribution in salt marshes. However, sympatric ostracod micro-crustaceans often co-occur within these salt marshes and can be found in dense populations, with field evidence suggesting exploitative competition for resources. Our experiments demonstrate resource limiting conditions caused significant increases in mosquito developmental times, decreased adult survival and decreased adult size. Overall, non-culicid exploitation experiments showed little effect on larval development and survival, but similar effects on adult size. We suggest that the alterations of adult traits owing to non-culicid controphic competition has potential to extend to vector-borne disease transmission.

Introduction

The effectiveness of exploitative competition for available food resources is driven by the presence of species within the same trophic level (controphic species) and species that share the same resources and functional feeding group [1, 2]. For mosquitoes, the presence of other species that are filter- and suspension-feeders can limit the amount of available food [3]. Consequently, the outcomes of controphic resource (exploitative) competition on mosquito development and survival should be equivalent to increased intra-specific resource limitation;

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with greater effects on mosquito development, survival and adult size with increasing competition. However, this hypothesis is yet to be investigated.

Over the last two decades, significant effort has been directed to understanding the diverse effects that biotic interactions have on the ecology of mosquito vectors [1, 4–7]. The larval life-stages of mosquitoes are the most sensitive to biotic interactions with impacts on developmental times, survival or changes to adult size. These impacts can, in turn, effect fecundity and, for vectors of disease, vector competence capacity [8–10]. To date, investigations have largely focused on larval predation, inter- and intra- specific mosquito competition (particularly with *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Say)) and mosquito oviposition behaviour as a means of predation and competition avoidance [11, 12]. The importance of competitive interactions with other non-culicid invertebrates, particularly non-culicid controphic species is poorly understood, yet may play an important role in mosquito abundance within natural conditions, with consequent implications for vectors [13, 14].

Across southern Australia *Ae. camptorhynchus* (Thomson) [15, 16] is a major vector of Ross River virus (RRV: Togaviridae: *Alphavirus*). Epidemiologically, RRV is Australia's most important vector-borne disease with clinical notifications ranging between 1451 – 7754 per annum, resulting in an annual economic impact of approximately $15 million dollars [17]. Salt marshes are particularly important habitats for this halo-tolerant vector due to the hyper-saline aquatic conditions. Such physiologically extreme environments result in lower aquatic species richness, and hence fewer predators and competitors of *Ae. camptorhynchus*, which is indicative of regions where this mosquito occurs across Australia [15, 18–20]. In these habitats *Ae. camptorhynchus* lays desiccation resistant eggs that undergo mass hatching with large pulses of rainfall or tidal inundations [21, 22]. Likewise, these pulses of water also result in high densities of micro-crustaceans that are capable of surviving dry periods [23, 24]. The *Diacypris* spp. (Crustacea: Ostracoda) that dominate the remaining aquatic fauna in these environments [25] are detritovores/herbivores, occupying the same functional feeding group as *Ae. camptorhynchus*. In addition, a negative density relationship from field evidence suggests these species may interact via exploitative competition [26]. If exploitative competition is occurring between these species we expect it will impact mosquito vector development, survival, abundance and size [26].

In this study we test the hypothesis that non-culicid controphic exploitative competition with ostracods reflects intra-specific resource limitation. The reciprocal of our hypothesis is that exploitative competition is not occurring between these taxa and ostracods will therefore have no impact on *Ae. camptorhynchus* development and survival. We examined our hypothesis using two experiments: an intra-specific resource limitation experiment with low densities of *Ae. camptorhynchus*; and a non-culicid controphic exploitative competition experiment with ostracods in increasing densities. Between the two experiments we contrast the changes in larval developmental times and survival, and effects on adult size. For both experiments, we predict decreased larval survival, increased development time, and reduced adult size as resources become limiting and controphic competition increases. We observed development and survival changes with intra-specific resource limitation and changes in adult size for both intra-specific resource limitation and controphic competition treatments. Our findings suggest no evidence of exploitative competition among *Ae. camptorhynchus* and ostracods during larval development, but detectable similarities to resource limitation with adult size.

Materials and Methods

Invertebrate collections

All mosquitoes and ostracods used in this study were sourced from the Primrose Sands salt marsh (147°39 East, 42°52 South), east of Hobart, Tasmania, whereby permission to access the

marsh was granted by the land owner. Water bodies found in the Primrose Sands salt marsh are very ephemeral, lasting on average only 14 days after inundation over the peak of summer [27]. Such environments are very depauperate of aquatic species diversity as shown by Carver *et.al.* [26] where, after rainfall, 89% of faunal abundance comprised of *Ae. camptorhynchus* (56%) and ostracods (33%), increasing to 91% in drying conditions (*Ae. camptorhynchus* 46% and ostracods 45%) [28]. Examination of the aquatic community strongly indicates that the ostracod, *Diacypris* spp. (Crustacea:Ostracoda) [25], is the only plausible competitor for resources with *Ae. camptorhynchus* and was, therefore, used in the competition experiment. Mosquitoes and ostracods were collected either after substantial rainfall or tidal inundations that submerged most of the salt marsh which provided newly hatched *Ae. camptorhynchus* larvae (1^{st} instars, < 24 hours old). While every attempt was made to collect early first mosquito instars, age was difficult to control in practice, so for consistency examination of developmental rates in this study are restricted to the second instar onwards. All invertebrates were collected using a 350 mL plastic larval dipper (Australian Entomological Supplies Pty. Ltd.).

Laboratory conditions

Following field collections, mosquito larvae were placed into 500 mL translucent cylindrical plastic containers with 200 mL of water at 35 ppt salinity ("Red Sea Salts"; at 35 ppt elements are 8.2–8.4 pH, 7.8–8.3 Alk (dKH), 420–440 Ca (mg/L), 1250–1310 Mg (mg/L) and 380–400 K (mg/L)), with containers placed randomly in temperature cabinets (Andrew Thorn Limited Qualtex 68 R4) at 23°C ± 0.05 S.E., 14:10 day/night. The temperature and salinity were chosen for laboratory conditions based on the average summer daily temperatures (°C) and salinity (ppt) of water bodies in the field near Hobart [27].

It is difficult to know the exact nutrient variables most relevant to mosquitoes within salt marshes and this is an important area for future studies. As a consequence, invertebrate food consisted of ground "Nutrafin Max Fish Flakes" (Pets Domain). Four grams were ground using a mortar and pestle and suspended in 1 L distilled water. At each feed the solution was agitated to allow for homogeneity and refrigerated at 6°C between feeds to standardise the potential growth of microbes. Although not entirely analogous to their field based diets, food levels and type were representative of other laboratory studies of culicid nutrition and development [29–31] and helped standardise nutritional quality and quantity which could otherwise vary if using field collected resources. New food was prepared fortnightly.

Experimental design

We conducted a paired experimental design to examine the effects of both intra-specific resource limitation and non-culicid exploitative competition on mosquito development and survival. While a fully crossed design would have been optimal, this was beyond the scope of the study owing to the logistics of available incubators and number of ostracods required. Instead this study provides a paired design where intra-specific resource limitation and non-culicid controphic exploitative competition experiments are contrasted. In the resource limitation experiment 50 larvae were exposed to one of four food resource levels (0.1 mL, 0.2 mL, 0.4 mL or 0.6 mL food/day), with six replicate cylinders per treatment. For the exploitative competition trials 50 larvae were exposed to one of four treatments of competitor (ostracod) density (0 (control), 150, 300 and 600 ostracods/cylinder). This reflects the observed range of ostracod densities per 350 mL larval dippers (Australian Entomological Supplies) in water bodies at Primrose Sands between 2011 and 2012 (1–2144, $n = 442$) [27]. Food resources remained at a constant level of 0.4 mL/day. Each treatment consisted of 10 replicate cylinders, with the non-culicid exploitation control treatment being comparable to the 0.4 mL/day treatment in

the resource experiment. Two replicates from each treatment from both experiments were dispersed evenly among three (resource experiment) or five (non-culicid exploitation experiment) independent incubators (akin to blocks), with the position of replicates randomised within each incubator.

Daily counts of mosquito larvae included the number of larvae, instar of each larva, number of pupae and number and sex of adults. Any mosquito larvae or pupae that died were recorded and removed from the container. All emergent mosquitoes were collected, sexed and their wings removed and mounted using water onto glass slides and sealed with clear nail varnish. The average length of left and right wings were used as a proxy for adult size [32]. These were measured at 25× magnification from the wing tip (excluding the fringe) to the arculus [10, 33] using Las EZ software (Leica Microsystems, Switzerland). Developmental time for instars–pupae in each replicate container was as the day at which 50% of surviving larvae reached the next stage of development.

To account for size variation of ostracods among treatments, a subsample of five ostracods from each controphic resource limiting replicate was removed at the beginning of the experiment and again at the end of the experiment (when the last mosquito emerged or died in each replicate container). Measurements of the carapace, from posterior to anterior, were conducted using an ocular micrometer (0.016mm units per graticule unit) on a Nikon SM2800 dissecting microscope at × 6.3 magnification. All remaining ostracods in each replicate were scored for survival.

Analyses

In both experiments we evaluated how treatment affected larval developmental times, survival, and adult size. Larval development was determined as developmental time when 50% of surviving larvae had reached the next each instar stage, pupa or adult. Survival was measured as the number of emerging adults and size was based on wing length measurements. An alternative approach to evaluating survival is by Cox hazard models, but we could not reliably identify individual survival in our experiment design, precluding this type of analysis. We used a Bayesian mixed effects modelling approach, with incubator as the random effect, owing to its superior ability to estimate coefficients than non-bayesian approaches, in a mixed effects framework [34]. In each model Y_{ij} (larval developmental time, survival and adult size) was measured for each replicat cylinder $i = 1,\ldots, n_j$ for incubator $j = 1,\ldots, k$. The distribution of Y among replicates was assumed to have a Gaussian distribution with parameter π_{ij}:

$$Y_{ij}|\,\pi_{ij} \sim \text{Gaussian}(\pi_{ij})$$

where π_{ij} is the modelled Y of replicate i in incubator j. We modelled the Y, π_{ij}, based on the effects of treatment

$$\pi_{ij} = \alpha_{ij} + \beta_{ji}x_i$$

where α and β are the model intercept and slope, respectively, for replicate i varying by incubator j, and x was the assigned experimental treatment (intra-specific resource or non- culicid controphic exploitative resource limiting level) for replicate i. An additional fixed effect of sex was included for the model of adult size. Prior distributions for all model parameters in the hierarchy (incubators) were given with the goal of providing conjugate priors that contain little to no influence on the posterior distributions of all the model parameters. We assumed normal

prior distributions on slopes, α, and intercepts, β, with mean μ and variance σ^2:

$$\alpha_j \sim \text{Normal}(\mu_\alpha, \sigma^2{}_\alpha), \text{ for } j = 1, \ldots, k$$

$$\beta_j \sim \text{Normal}(\mu_\beta, \sigma^2{}_\beta), \text{ for } j = 1, \ldots, k$$

For the variance parameters, σ^2, we determined and utilized non-informative uniform prior hyper-parameter distributions, specified as σ^2~Uniform (0, 100), which was used across all models. Models were fitted in R (v 3.0.3) [35] using the 'MCMCglmm' package [36], with MCMC chains run for 13,000 iterations after a burn-in period of 3,000 iterations, ensuring convergence of model parameters, assessed following Gelman and Hill [34]. We summarized posterior distributions of model coefficients, β, by the Bayesian median and 95% credible intervals and MCMC simulated *P*-values.

We assessed consistency of results between the two experiments at the 0.4 mL/day intra-specific resource limiting treatment and the control treatment (no ostracods and same food level) from the non-culicid resource exploitation experiment. This was undertaken for development, survival and adult size using a Bayesian mixed effects model as described above, but with experiments being the fixed effect.

Using a Bayesian mixed effects model as previously described, we also investigated ostracod size (measured through sub-samples) and mortality amongst competition treatments (end count of ostracods) to explore if these factors changed *Ae. camptorhynchus* developmental times, survival and size or ostracod development and mortality. This analysis was undertaken for quality control purposes, as changes in these could confound non-culicid exploitative competition treatment effects.

Results

Development time

As the amount of intra-specific resources increased the time it took for *Ae. camptorhynchus* to develop decreased (**Table 1**). This relationship was observable within each larval stage with the exception of the pupal stage when mosquitoes do not feed. Overall, the mean developmental time of *Ae. camptorhynchus* to adult was 35 days with a range of 21–70 days (**Fig 1**; S1 Table). In contrast, the time taken for *Ae. camptorhynchus* larvae to develop was unrelated to the number of competitors. The only significant effect detected was for third instar which developed more slowly with increasing numbers of ostracods (**Table 1**). Mean time to develop to adult was 27 days ranging between of 33–39 days (Fig 1; S1 Table). When comparing the two experiments, there was no difference in developmental time between the 0.4mL/day intra-specific resource limitation treatment and the control treatment (no ostracods and same food level) for the non-culicid exploitation competition experiment ($p = 0.176$) (Fig 1).

Survival

Aedes camptorhynchus survival decreased as intra-specific resources became more limited (Table 1). The mean range of survival was between 61.4% – 96.1% (Fig 1; S1 Table). By comparison, increasing non-culicid exploitative competition did not affect *Ae. camptorhynchus* survival (**Table 1**), with a mean range of survival between 60.8% – 72.6% (**Fig 1**; S1 Table). Comparing the two experiments survival was slightly higher in the resource limitation than the control treatment (no ostracods and same food level) for non-culicid exploitative competition experiments ($p = 0.028$) (**Fig 1**).

Table 1. Bayesian mixed-effects regression results showing effects (median coefficient and 95% credible intervals) of both resource limitation and exploitation competition treatments on *Ae. camptorhynchus* larval development time (in days) between instar, pupae and total developmental time to adulthood, the effect of treatment on survival and the effect of both treatment and sex on adult wing length (mm) for both resource limitation and competition experiments. Significant *p* values are in bold.

Experiment	Resources				Competition			
	coefficient	2.5%CI	97%CI	p	coefficient	2.5%CI	97%CI	p
				Development (days)				
Treatment effect on larvae								
Second instar	-0.710	-1.169	-0.189	**0.006**	-4.74e-5	-2.15e-3	1.55e-3	0.984
Third instar	-3.379	-4.594	-1.789	**<0.001**	0.002	0.260–3	0.003	**0.008**
Fourth instar	-1.431	-2.405	-0.293	**0.004**	0.003	-0.002	0.008	0.262
Pupae	0.036	-0.137	0.207	0.654	0.002	-0.003	0.008	0.398
Overall	-6.893	-8.658	-5.205	**<0.001**	0.003	-0.007	0.013	0.55
				Survival				
Treatment	11.257	8.088	14.201	**<0.001**	0.006	-0.024	0.036	0.696
				Wing length (mm)				
Treatment	0.069	0.061	0.077	**<0.01**	<-0.001	<-0.001	<-0.001	**<0.01**
Sex	0.038	0.007	0.068	**0.02**	-0.093	-0.037	0.017	0.526

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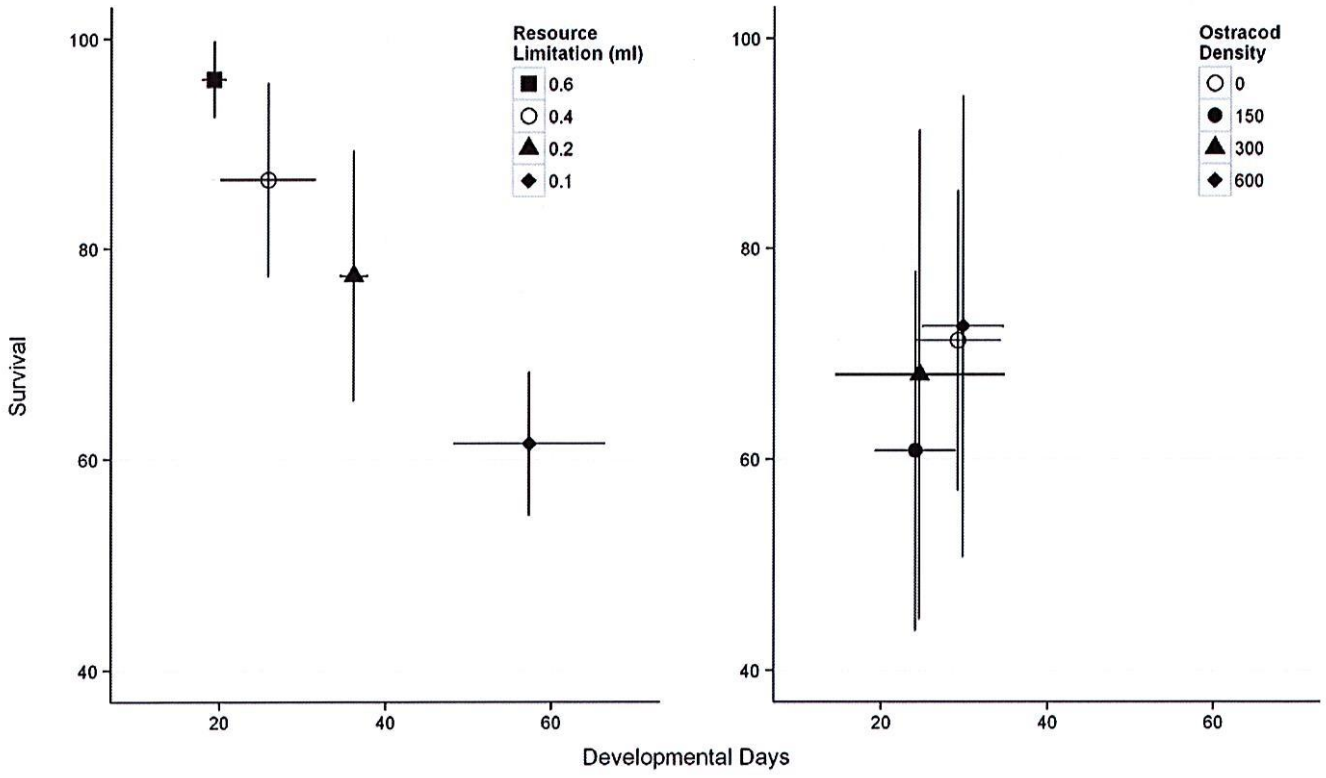

Fig 1. Effects of resource limitation (left panel) and exploitation competition (right panel) on *Ae. camptorhynchus* mean development time and survival to adulthood (mean ± SD). Treatments with open symbols are directly comparable.

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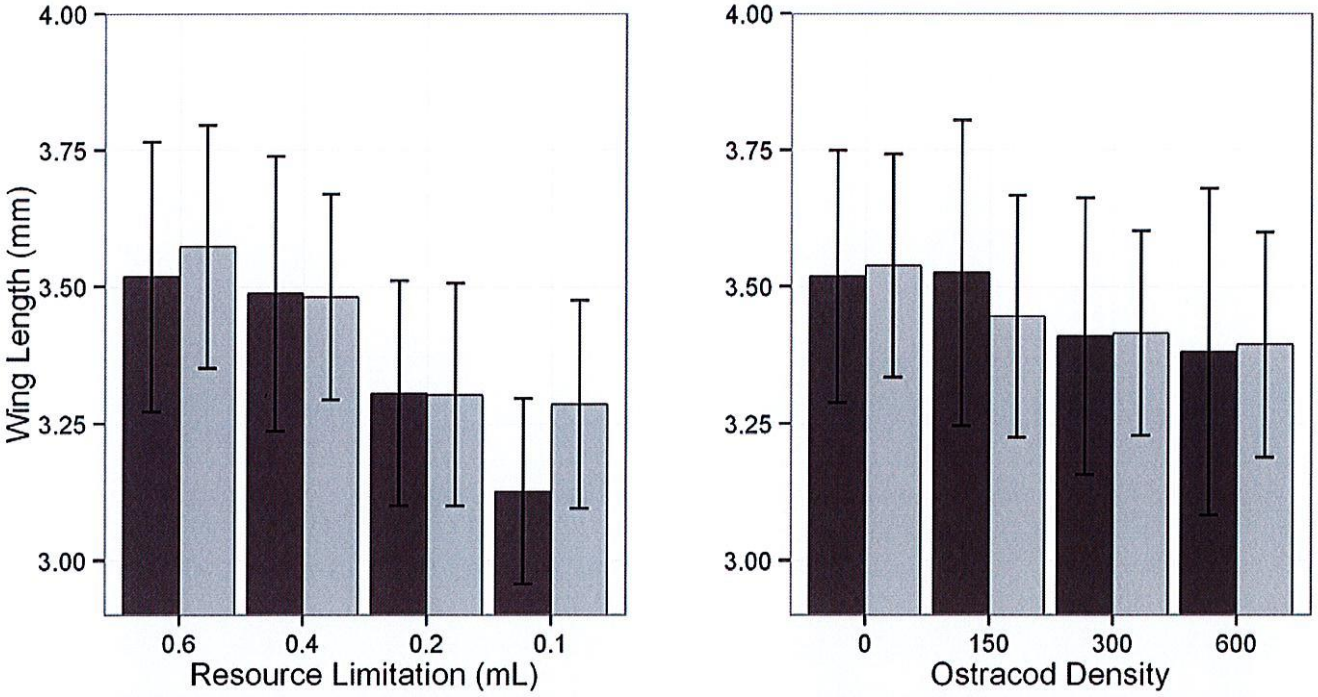

Fig 2. Mean (± SD) wing length (mm) for *Ae. camptorhynchus* adult females (dark grey) and males (light grey) for both resource limitation (left panel) and competition (right panel) treatments.

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Adult size

The size of *Ae. camptorhynchus* (based on wing length) declined as intra-specific resources became more limiting (Table 1). Overall, emerging adults were 9.86% larger in the most resource rich treatment, relative to all the other resource treatments (Fig 2). There was also a sex-specific effect on size with the wings of males being 0.15 mm larger than females, however this was only in the most limiting resource treatment. Likewise, increased non-culicid controphic competitors resulted in decreased size of *Ae. camptorhynchus* adults (Table 1). On average adults emerging from the control (no ostracods) were 4% larger than adults from the highest ostracod density treatment (Fig 2). There was no significant sex-specific size difference between treatments owing to ostracods (Table 1). Comparing the two experiments, adult size did not differ ($p = 0.91$, Fig 2).

Ostracod mortality and size

Ostracods within the non-culicid controphic exploitative treatments averaged 0.842 mm in length with no significant difference in size over the duration of the experiment ($P = 0.624$). Similarly, ostracod survival did not significantly differ between experimental treatments ($P = 0.628$), with an overall average 17% loss of ostracods.

Discussion

Like resource limitation, competitive interactions can affect mosquito life history, thereby potentially having an important role in the ecology of disease vectors. The results of our intra-specific resource limitation experiments are consistent with previous work that has investigated

the effects of resource limitation on mosquito development and survival [31, 37–40] showing decreased developmental times, decreased survival and reduced adult sizes as resources become more scarce. However, when numbers of ostracod controphic competitors are increased, at least in our experimental conditions, exploitative competition had no impact on larval *Ae. camptorhynchus* developmental traits, but do effect adult size. Adult body size decreased as the density of ostracods increased, suggesting exploitative competition among *Ae. camptorhynchus* and ostracods may cause some effects similar to resource limitation. Our study is the first to directly test non-culicid controphic competition with ostracods, which form the same functional feeding group as *Ae. camptorhynchus*. We demonstrate that this interaction is an important component to mosquito ecology providing insight into the complexity of aquatic interactions with an important vector species.

As expected, reducing the resources available to *Ae. camptorhynchus* drove increased developmental times [31, 38–41] likely through intra-specific competition. Similar extension of developmental time has been demonstrated for both *Ae. albopictus* (Skuse) and *Ae. aegypti* in limiting diets and this was inferred to indicate impacts on accumulation, assimilation and storage of energy gained from food resources [42]. Prolonged larval developmental times could have negative impacts in the field, especially in conditions where water bodies are extremely ephemeral such as salt marshes. For example, extended developmental durations in these aquatic habitats increase exposure to habitat loss through water bodies drying up [43], thereby having the potential to result in decreased population sizes and reduced disease transmission.

The effects on non-culicid controphic competition on mosquitoes is a relatively new field of research, with few studies detailing increased developmental times in the presence of such exploitative competitors [1, 4, 44]. In these situations, where resources are shared [1, 3, 24], it is common to expect exploitation competition of one species over the other [45, 46]. It was expected that our non-culicid exploitative experiment would show that increasing ostracod density would cause longer *Ae. camptorhynchus* development. This expectation was supported by other studies, such as Stav et al. [44] where the authors presented increased time to metamorphosis of *Culex pipens* (Linnaeus) in the presence of *Daphnia magna* (Straus). In our trials the only significant impact on development we observed was during the third instar, with no effect of ostracod density on overall developmental times. While it is plausible that the densities within our trials were inadequate to result in exploitative competitive outcomes on *Ae. camptorhynchus* life history, the number of ostracods used in these trials reflect ostracod densities found in natural water bodies [26, 27]. Therefore, given that *Ae. camptorhynchus* maintained consistent rates of development across density treatments of ostracods, and ostracod mortality was not significant across treatments, we suggest exploitative competition is unlikely to be limiting larval development in our system.

Similarly, our intra-specific resource limiting trials showed *Ae. camptorhynchus* survival declined as resources became limited, but *Ae. camptorhynchus* survival did not change with increasing densities of ostracod competitors. Declines in mosquito survival has been documented in other non-culicid controphic competitive studies [1, 13], for example, Mokany and Shine [13] demonstrated a decline in *Aedes australis* survival as a result of interference competition between tadpoles (*Limnodynastes peronii*), however this was a result of chemical interference not exploitative competition. In contrast, Daugherty and Juliano [47] demonstrated improved survival of *Ae. triseriatus* in the presence of higher densities of scirtid beetles, because of the quantities of faeces excreted by the scirtids supported microorganisms that nourished the developing mosquito larvae. This, again, is not exploitative competition. Overall, our results suggest that, like developmental rates, the density of ostracods has little impact on mosquito survival through exploitative competition in this system.

Given the lack of detectable effects of ostracod density on *Ae. camptorhynchus* larval development and survival, it was somewhat surprising to see an effect on adult size. Limiting resources is known to cause emerging mosquitoes to be smaller [31]. For example, *Ae. agypti* adults emerging from nutrient deprived crowded conditions were significantly smaller. Here we suggest ostracod competitors may have a similar effect. Therefore, detecting the effects of competition between ostracods and *Ae. camptorhynchus* may be most sensitively measured through adult size rather than larval development and survival. On the other hand, it is possible that there is a coexistence between *Ae. camptorhynchus* and ostracods in these habitats and consequentially partitioning of resources driven by differences in species' niches [45] and that the changes in adult size observed in these trials were driven by intra-specific competition, although further investigation is necessary.

Even though adult size was negatively affected by intra-specific resource limitation, males were significantly larger than females for the most limiting resource treatment. It is known that larval females take longer to develop as a trade-off for greater accumulation of resources which ultimately results in larger sizes and improved fecundity [48, 49]. However, under increased non-culicid exploitative resource limitation, there may be an effect on female *Ae. camptorhynchus* reproductive success. In fact, resource limitation is correlated with fewer oogenesis cycles resulting in reduced fecundity [50] and a decreased ability of mosquitoes to carry and transmit disease [8, 31, 41, 51]. Due to *Ae. camptorhynchus* being anautogenous [52] thereby requiring a blood meal to complete oogenesis, it is likely that this reduction in size for *Ae. camptorhynchus* limits vectorial abilities, although further research is necessary to understand the connection with *Ae. camptorhynchus* size and the ability to transmit RRV. A potential caveat is that experimental adults would not represent field adults, however, we have found substantial overlap in mean wing length between field caught adults and experimental adults (competition, 3.46 ±0.27, resources, 3.37 ± 0.26 and field, 3.60 ± 0.44).

Overall our experiments suggest exploitative competition between *Ae. camptorhynchus* and ostracods is limited in laboratory conditions. It is possible that environmental conditions may be more important than competition alone in that competitive effects may change given different abiotic conditions [2, 53, 54]. Such changes can be observed when habitats are drying out or with the addition of new invertebrates through rainfall or tide [55]. For example, hatching of first instar larvae may differ to ostracods hatching from dormancy [56, 57]. Such a situation could result in a time window in which first instar mosquitoes have a competitive advantage both in size and nutrient acquisition. It might also be possible that different life stages of *Ae. camptorhynchus* larvae are more sensitive to environmental changes or intra-specific competition [58]. Therefore, replicating these experiments in natural conditions in the field, with the addition of a fully crossed design (where all levels of resource limitation are also tested with ostracod densities against *Ae. camptorhynchus* development, survival and size) would benefit our understanding on the relationship between *Ae. camptorhynchus* inter- and intra-specific interactions and provide further insight on the complexity of competitive systems.

A limitation of our study is that we did not measure adult survival. Epidemiological models of mosquito-borne disease transmission incorporate adult longevity which can have significant effects on disease transmission [59]. However, environmental effects on larval development and survival have been demonstrated to be both condition- and species-specific, with strong associations between adult longevity, developmental times and body size demonstrated for some species [59, 60]. While resource limitation had a greater impact on *Ae. camptorhynchus* survival and development, it is possible that densities of non-culicid competitors (and resource limitation) may result in reduced adult longevity especially considering both treatments had an effect on adult size.

We demonstrate that intra-specific resource limitation and controphic competition have a direct impact on adult sizes, however, changes to *Ae. camptorhynchus* life history in exploitation competition environments is not so obvious. We conclude that controphic competition, although quite complex, may have a role in influencing vector-borne disease and implications to human health.

Supporting Information

S1 Table. Mean and maximum developmental time and survival for *Ae. camptorhynchus* larvae by treatment for both resource limitation and competition experiments.
(PDF)

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Author Contributions

Conceived and designed the experiments: RR GRA SC LAB. Performed the experiments: RR DF. Analyzed the data: SC LAB RR. Wrote the paper: RR SC GRA. Editorial advice: LAB PW.

References

1. Blaustein L, Chase JM. Interactions between mosquito larvae and species that share the same trophic level. Annual Review of Entomology. 2007; 52:489–507. PMID: 16978142
2. Juliano SA. Species Interactions Among Larval Mosquitoes: Context Dependence Across Habitat Gradients. Annual Review of Entomology. 2009; 54:37–56. doi: 10.1146/annurev.ento.54.110807.090611 PMID: 19067629
3. Merritt RW, Dadd RH, Walker ED. Feeding behaviour, natural food, and nutritional relationships of larval mosquitos. Annual Review of Entomology. 1992; 37:349–76. PMID: 1347208
4. Knight TM, Chase JM, Goss CW, Knight JJ. Effects of interspecific competition, predation, and their interaction on survival and development time of immature Anopheles quadrimaculatus. Journal of Vector Ecology. 2004; 29(2):277–84. PMID: 15707287
5. Juliano SA. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? Ecology. 1998; 79(1):255–68.
6. Beketov MA, Liess M. Predation risk perception and food scarcity induce alterations of life-cycle traits of the mosquito *Culex pipens*. Ecological Entomology. 2007; 32(4):405–10.
7. Werner AK, Goater S, Carver S, Robertson G, Allen GR. Environmental drivers of Ross River virus in southeastern Tasmania, Australia; towards strengthening public health interventions. Epidemiology and Infection. 2012; 140(2):359–71. doi: 10.1017/S0950268811000446 PMID: 21439102
8. Nasci RS, Mitchell CJ. Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. Journal of Medical Entomology. 1994; 31(1):123–6. PMID: 8158614
9. Mokany A, Shine R. Competition between tadpoles and mosquitoes: the effects of larval density and tadpole size. Australian Journal of Zoology. 2002; 50(5):549–63.
10. Schneider JR, Chadee DD, Mori A, Romero-Severson J, Severson DW. Heritability and adaptive phenotypic plasticity of adult body size in the mosquito *Aedes aegypti* with implications for dengue vector competence. Infection, Genetics and Evolution. 2011; 11(1):11–6. doi: 10.1016/j.meegid.2010.10.019 PMID: 21070891
11. Eitam A, Blaustein L. Oviposition habitat selection by mosquitoes in response to predator (Notonecta maculata) density. Physiological Entomology. 2004; 29(2):188–91.

12. Mokany A, Shine R. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. Austral Ecology. 2003; 28(1):33–7.

13. Mokany A, Shine R. Competition between tadpoles and mosquito larvae. Oecologia. 2003; 135 (4):615–20. PMID: 12684864

14. Duquesne S, Kroeger I, Kutyniok M, Liess M. The potential of cladocerans as controphic competitors of the mosquito *Culex pipiens*. Journal of Medical Entomology. 2011; 48(3):554–60. PMID: 21661316

15. Russell RC. Ross river virus; ecology and distribution. Annual Review of Entomology. 2002; 47:1–31. PMID: 11729067

16. Ballard JWO, Marshall ID. An investigation of the potential of *Aedes camptorhynchus* (Thom) as a vector of Ross River virus. Australian Journal of Experiemntal Biology and Medical Science. 1986; 64:197–200.

17. Aaskov J, Fokine A, Liu WJ. Ross River virus evolution: implications for vaccine development. Future Virol. 2012; 7(2):173–8.

18. Carver S, Spafford H, Storey A, Weinstein P. The Roles of Predators, Competitors, and Secondary Salinization in Structuring Mosquito (Diptera: Culicidae) Assemblages in Ephemeral Water Bodies of the Wheatbelt of Western Australia. Environ Entomol. 2010; 39(3):798–810. doi: 10.1603/EN09235 PMID: 20550792

19. Chapman HF, Hughes JM, Jennings C, Kay BH, Ritchie SA. Population structure and dispersal of the saltmarsh mosquito Aedes vigilax in Queensland, Australia. Medical and Veterinary Entomology. 1999; 13(4):423–30. PMID: 10608232

20. Van Schie C, Spafford H, Carver S, Weinstein P. Salinity tolerance of *Aedes camptorhynchus* (Diptera: Culicidae) from two regions in southwestern Australia. Australian Journal of Entomology. 2009; 48 (4):293–9.

21. Bader CA, Williams CR. Eggs of the Australian saltmarsh mosquito, *Aedes camptorhynchus*, survive for long periods and hatch in instalments: implications for biosecurity in New Zealand. Medical and Veterinary Entomology. 2011; 25(1):70–6. doi: 10.1111/j.1365-2915.2010.00908.x PMID: 20840222

22. Carver S, Spafford H, Storey A, Weinstein P. Colonization of ephemeral water bodies in the wheatbelt of Western Australia be assemblages of mosquitoes (Diptera: Culicidae): Role of environmental factors, habitat and disturbance. Environ Entomol. 2009; 38(6):1585–94. PMID: 20021752

23. Williams DD. Temporary ponds and their invertebrate communities. Aquatic Conservation: Marine and Freshwater Ecosystems. 1997; 7(2):105–17.

24. Rossi V, Benassi G, Belletti F, Menozzi P. Colonization, population dynamics, predatory behaviour and cannibalism in *Heterocypris incongruens* (Crustacea: Ostracoda). Journal of Limnology. 2011; 70 (1):102–8.

25. Walsh R. Australian Waterlife. Aquatic Micro-invertebrate Ecologist, Freshwater Ecology & Limnology ed: Available: www.australianwaterlife.com.au; 2015.

26. Carver S, Goater S, Allen GR, Parr R, Fearnley E, Weinstein P. Relationships of the Ross River Virus (Togoviridae: *Alphavirus*) vecor, *Aedes camptorhynchus* (Thomson) (Diptera:Culicidae), to biotic and abiotic factors in saltmarshes of south-eastern Tasmania, Australia: a preliminary study. Australian Journal of Entomology. 2011; 50:344–55.

27. Rowbottom R, Carver S, Barmuta LA, Weinstein P, Allen GR. The abiotic/biotic predictor(s) that drive *Aedes camptorhynchus* Thomson (Diptera: Culicidae) populations in RRV regions of Tasmania. [Thesis]. In press *unpublished data*.

28. Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, et al. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature. 2010; 468(7324):647–52. doi: 10.1038/nature09575 PMID: 21124449

29. Farjana T, Tuno N, Higa Y. Effects of temperature and diet on development and interspecies competition in *Aedes aegypti* and *Aedes albopictus*. Medical and Veterinary Entomology. 2012; 26(2):210–7. doi: 10.1111/j.1365-2915.2011.00971.x PMID: 21781139

30. Tun-Lin W, Burkot TR, Kay BH. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Medical and Veterinary Entomology. 2000; 14(1):31–7. PMID: 10759309

31. Mitchell-Foster K, Ma BO, Warsame-Ali S, Logan C, Rau ME, Lowenberger C. The influence of larval density, food stress, and parasitism on the bionomics of the dengue vector *Aedes aegypti* (Diptera: Culicidae): implications for integrated vector management. Journal of Vector Ecology. 2012; 37 (1):221–9. doi: 10.1111/j.1948-7134.2012.00220.x PMID: 22548557

32. Lounibos LP. Geographical and developmental components of adult size of neotropical Anopheles (Nyssorhynchus). Ecological Entomology. 1994; 19(2):138–46.

33. Packer MJ, Corbet PS. Size variation and reproductive success of female *Aedes punctor* (Diptera: Culicidae). Ecological Entomology. 1989; 14(3):297–309.

34. Gelman A, Hill J. Data Analysis Using Regression and Multilevel/Hierarchial Models: Cambridge University Press; 2006.

35. R. The R project for Statistical Computing. [cited 2014]; Available: http://www.R-project.org/.

36. Hadfield JD. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software. 2010; 33(2):22.

37. Juliano SA. Population dynamics. Journal of the American Mosquito Control Association. 2007; 23 (2):265–75. PMID: 17853611

38. Bevins SN. Timing of resource input and larval competition between invasive and native container-inhabiting mosquitoes (Diptera: Culicidae). Journal of Vector Ecology. 2007; 32(2):252–62. PMID: 18260515

39. Gilles JRL, Lees RS, Soliban SM, Benedict MQ. Density-Dependent Effects in Experimental Larval Populations of *Anopheles arabiensis* (Diptera: Culicidae) Can Be Negative, Neutral, or Overcompensatory Depending on Density and Diet Levels. Journal of Medical Entomology. 2011; 48(2):296–304. PMID: 21485365

40. Padmanabha H, Bolker B, Lord CC, Rubio C, Lounibos LP. Food Availability Alters the Effects of Larval Temperature on *Aedes aegypti* Growth. Journal of Medical Entomology. 2011; 48(5):974–84. PMID: 21936315

41. Jennings CD, Kay BH. Dissemination barriers to Ross River virus in *Aedes vigilax* and the effects of larval nutrition on their expression. Medical and Veterinary Entomology. 1999; 13(4):431–8. PMID: 10608233

42. Barrera R. Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. Ecological Entomology. 1996; 21(2):117–27.

43. Knight J. A Model of Mosquito–Mangrove Basin Ecosystems with Implications for Management. Ecosystems. 2011; 14(8):1382–95.

44. Stav G, Blaustein L, Margalit Y. Individual and interactive effects of a predator and controphic species on mosquito populations. Ecological Applications. 2005; 15(2):587–98.

45. Amarasekare P. Competitive coexistence in spatially structured environments: a synthesis. Ecology Letters. 2003; 6(12):1109–22.

46. Morin PJ. Community ecology / Peter J. Morin: Malden, Mass.; Oxford: Blackwell Science.; 1999.

47. Daugherty MP, Juliano SA. Leaf Scraping Beetle Feces Are a Food Resource for Tree Hole Mosquito Larvae. American Midland Naturalist. 2003; 150(1):181–4.

48. Blackmore MS, Lord CC. The relationship between size and fecundity in *Aedes albopictus*. Journal of Vector Ecology. 2000; 25:212–7. PMID: 11217219

49. Clements AN. The Biology of Mosquitoes, Volume 1. New York: Chapman and Hall; 1992.

50. Telang A, Li Y, Noriega FG, Brown MR. Effects of larval nutrition on the endocrinology of mosquito egg development. Journal of Experimental Biology. 2006; 209(4):645–55.

51. Muturi EJ, Kim C-H, Alto BW, Berenbaum MR, Schuler MA. Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. Tropical Medicine & International Health. 2011; 16(8):955–64.

52. Barton PS, Aberton JG. Larval development and autogeny in *Ochlerotatus camptorhynchus* (Thomson) (Diptera: Culicidae) from southern Victoria. Proceedings of the Linnean Society of New South Wales. 2005; 126(1):261–7.

53. Tilman D. Resource competition and community structure / David Tilman: Princeton, N.J. Princeton University Press, 1982.; 1982.

54. Costanzo KS, Kesavaraju B, Juliano SA. Condition-specific competition in container mosquitoes: The role of noncompeting life-history stages. Ecology. 2005; 86(12):3289–95. PMID: 17450188

55. Chase JM, Knight TM. Drought-induced mosquito outbreaks in wetlands. Ecology Letters. 2003; 6:1017–24.

56. Khatchikian CE, Dennehy JJ, Vitek CJ, Livdahl TP. Environmental effects on bet hedging in *Aedes* mosquito egg hatch. Evol Ecol. 2010; 24(5):1159–69.

57. Campos RE, Sy VE. Variation in the hatching response of *Ochlerotatus albifasciatus* egg batches (Diptera: Culicidae) in temperate Argentina. Memorias Do Instituto Oswaldo Cruz. 2006; 101(1):47–53. PMID: 16699709

58. Silberbush A, Tsurim I, Rosen R, Margalith Y, Ovadia O. Species-Specific Non-Physical Interference Competition among Mosquito Larvae. PLoS ONE. 2014; 9(2):e88650. doi: 10.1371/journal.pone.0088650 PMID: 24558406

59. Reiskind MH, Lounibos LP. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Medical and Veterinary Entomology. 2009; 23(1):62–8. doi: 10.1111/j.1365-2915.2008.00782.x PMID: 19239615

60. Mogi M, Miyagi I, Abadi K, Syafruddin. Inter- and Intraspecific Variation in Resistance to Desiccation by Adult *Aedes* (Stegomyia) spp. (Diptera: Culicidae) from Indonesia. Journal of Medical Entomology. 1996; 33(1):53–7. PMID: 8906905